

PCT/NZ2004/000275

REC'D 17 NOV 2004

WIPO PCT

CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 28 September 2004 with an application for Letters Patent number 535618 made by Auckland UniServices Limited and William Robert Wilson.

Dated 8 November 2004.

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

Neville Harris

Neville Harris
Commissioner of Patents, Trade Marks and Designs



Patents Form No. 4

Our Ref: JC223089

Patents Act 1953

PROVISIONAL SPECIFICATION

NOVEL NITROPHENYL MUSTARD AND NITROPHENYLAZIRIDINE
ALCOHOLS AND THEIR CORRESPONDING PHOSPHATES AND THEIR
USE AS BIOREDUCTIVE DRUGS

We, **Auckland UniServices Limited**, a New Zealand company, of Level 10, 70 Symonds Street, Auckland 1001 New Zealand and **William Robert Wilson**, a New Zealand citizen of 199 Smith Road, RD2 Waiuku, New Zealand do hereby declare this invention to be described in the following statement:

NOVEL NITROPHENYL MUSTARD AND NITROPHENYLAZIRIDINE
ALCOHOLS AND THEIR CORRESPONDING PHOSPHATES AND THEIR USE AS
BIOREDUCTIVE DRUGS

- 5 The present invention relates to novel nitrophenyl mustard and nitrophenylaziridine alcohols, to their corresponding phosphates, to their use as anticancer drugs; as bioreductive drugs in hypoxic tumours, and to their use in gene-directed enzyme-prodrug therapy (GDEPT) and antibody-directed enzyme-prodrug therapy (ADEPT), in conjunction with nitroreductase enzymes.

10

Background to the invention

- 15 The use of tumour-selective prodrugs (relatively inactive compounds that can be selectively converted to more active compounds *in vivo*) is a valuable concept in cancer therapy (see, for example Denny, *Eur. J. Med. Chem.* (2001) 36, 577).

- 20 For example a prodrug may be converted into an anti-tumour agent under the influence of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour associated antigen. The combination of such a prodrug with such an enzyme monoclonal/antibody conjugate represents a very powerful clinical agent. This approach to cancer therapy, often referred to as "antibody directed enzyme/prodrug therapy" (ADEPT), is disclosed in W088/07378.

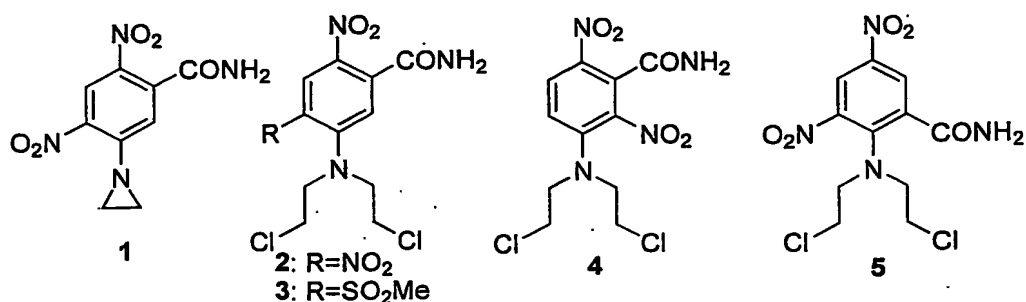
- 25 A further therapeutic approach termed "virus-directed enzyme prodrug therapy" (VDEPT) has been proposed as a method for treating tumour cells in patients using prodrugs. Tumour cells are targeted with a viral vector carrying a gene encoding an enzyme capable of activating a prodrug. The gene may be transcriptionally regulated by tissue specific promoter or enhancer sequences. The viral vector enters tumour cells and expresses the enzyme, in order that a prodrug is converted to an active drug within the tumour cells (Huber et al., *Proc. Natl. Acad. Sci. USA* (1991) 88, 8039). Alternatively, non-viral methods for the delivery of genes have
30 been used. Such methods include calcium phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-mediated DNA transfer. These are reviewed in

Morgan & French, *Annu. Rev. Biochem.*, 1993, 62; 191. The term "GDEPT" (gene-directed enzyme prodrug therapy) is used to include both viral and non-viral delivery systems.

4-Nitroaromatic compounds are reduced by both mammalian and bacterial flavoprotein enzymes, which effect stepwise addition of up to six electrons. The major enzymatic metabolite is usually the 4-electron reduced species (hydroxylamine).

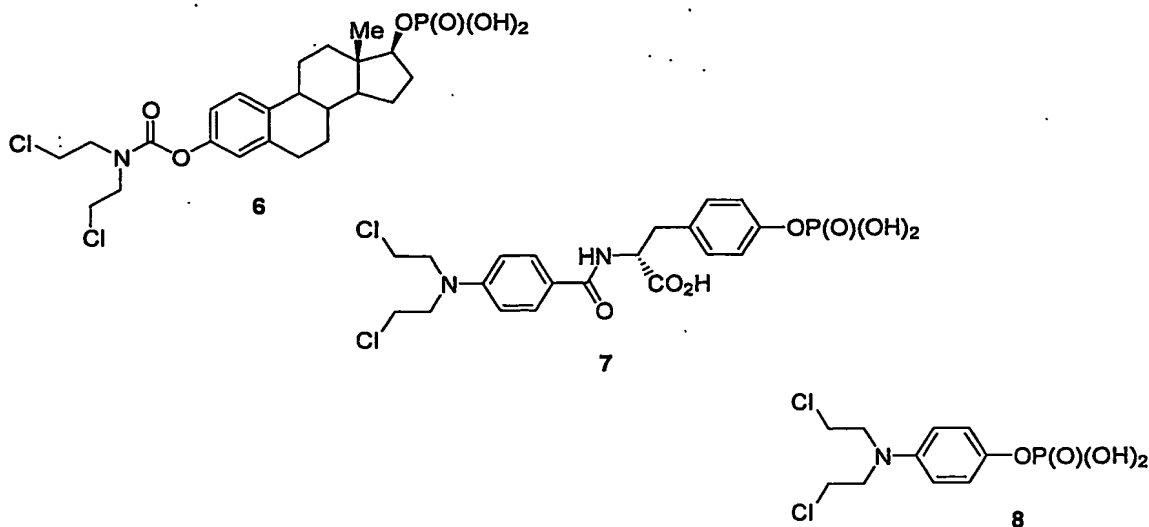
A number of nitrophenyl mustards and nitrophenylaziridines have been reported as prodrugs for use in gene-directed enzyme-prodrug therapy (GDEPT) in conjunction with nitroreductase enzymes. In particular, CB 1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide; (1) [shown below] is reported to be a substrate for the aerobic nitroreductase NTR (nfsB gene product) isolated from *E. coli* B (Boland et al., *Biochem. Pharmacol.* 1991, 41, 867-875; Anlezark et al., *Biochem. Pharmacol.* 1992, 44, 2289-2295; Parkinson et al., *J. Med. Chem.* 2000, 43, 3624). This compound has been used as a prodrug in both ADEPT (Knox et al., *Biochem. Pharmacol.*, 1995, 49, 1641-1647) and GDEPT (Bridgewater et al., *Eur. J. Cancer*, 1995, 31A, 2362-2370; Bailey et al., *Gene Ther.*, 1996, 3, 1143-1150; Bailey and Hart, *Gene Ther.*, 1997, 4, 80-81; Green et al., *Cancer Gene Ther.*, 1997, 4, 229-238) applications, including a clinical trial (Chung-Faye et al., *Clin. Cancer Res.*, 2001, 7, 2662-2668).

Similarly, the dinitrophenyl mustard SN 23862 (2) is also a substrate for NTR, and shows selective toxicity towards cell lines that express the enzyme. It is activated by nitro group reduction (Palmer et al., *J. Med. Chem.*, 1995, 38, 1229; Kestell et al., *Cancer Chemother. Pharmacol.*, 2000, 46, 365-374). The 4-SO₂Me derivative (3) was also a substrate (Atwell et al., *Anti-Cancer Drug Des.*, 1996, 11, 553), as were the regioisomers (4) and (5) (Friedlos et al., *J. Med. Chem.*, 1997, 40, 1270).



However, compounds of this type were not very effective as bioreductive prodrugs when these compounds were activated in hypoxic tumour tissue by endogenous reductase enzymes, showing potency ratios of 2-5 fold under hypoxic conditions relative to oxic conditions in the wild-type AA8 cell line, using a clonogenic assay (Palmer et al., J. Med. Chem. 1996, 39, 2518-2528).

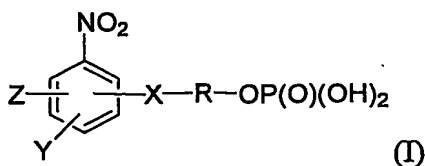
Some phosphate analogues of mustards have been described, for the purpose of solubilising the compounds. The best known is estramustine phosphate (Estracyt; 6), which has been shown to bind to tubulin binding domains on various microtubule-associated proteins (Moraga et al., Biochim. Biophys. Acta, 1992, 1121, 97-103), and which has been shown to be active in advanced breast cancer (Keren-Rosenberg et al., Semin. Oncol., 1997, 24 (Suppl. 3), 26-29), but has not been shown to be activated by NTR or hypoxia. Another study has also shown estramustine phosphate to be a radiation sensitizer (Kim et al., Int. J. Radiat. Oncol. Biol. Phys., 1994, 29, 555-557). The phenol mustard phosphate analogue 7 is a carboxypeptidase substrate that is not activated under hypoxic conditions, (Matsui et al., Japanese Patent 07082280 A2, 1995), and the solubilised mustard 8 has been described as a phosphatase inhibitor but has not been shown to be activated under hypoxic conditions (Workman, Chem.-Biol. Interact., 1978, 20, 103-112).



It is an object of the present invention to provide a specific class of nitrophenyl mustards and aziridines, bearing short-chain alcohols, and their corresponding phosphates, as anticancer drugs or bioreductive prodrugs or to at least provide the public with a useful alternative.

Summary and detailed description

In a first aspect, the present invention provides novel phosphate compounds of Formula 1



wherein:

X may represent at any available ring position $-\text{CONH}-$, $-\text{SO}_2\text{NH}-$, $-\text{O}-$, $-\text{CH}_2-$, $-\text{NHCO}-$ or $-\text{NHSO}_2-$;

R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

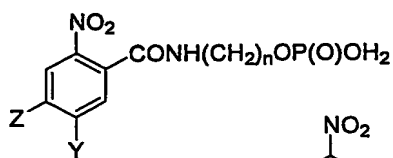
Y may represent at any available ring position $-\text{N}$ -aziridiny, $-\text{N}(\text{CH}_2\text{CH}_2\text{W})_2$ or

$-\text{N}(\text{CH}_2\text{CHMeW})_2$, where each W is independently selected from halogen or $-\text{OSO}_2\text{Me}$.

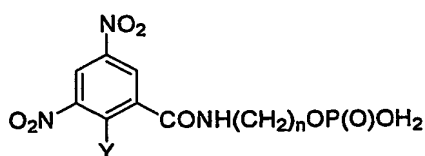
Z may represent at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

and pharmaceutically acceptable salts and derivatives thereof.

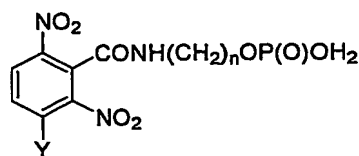
- 5 In a preferred embodiment, the phosphate compound of Formula (I) is selected from a compound represented by formulae (Ia), (Ib) or (Ic)



(Ia)

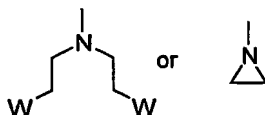


(Ib)



(Ic)

wherein Y may represent



and wherein

- 10 n may represent 1 to 6

Z may represent -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

where each W is independently selected from halogen or -OSO₂Me

and pharmaceutically acceptable salts and derivatives thereof.

- 15 Preferably, the phosphate compound of Formula I is selected from the following:

2-[[2-[Bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

3-[[5-[Bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

3-[[5-[Bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate;

2-[[2-[Bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate;

- 20 2-[(2-Chloroethyl)-2,4-dinitro-6-[[[2-(phosphonoxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-({2-[Bis(2-bromopropyl)amino]-3,5-dinitrobenzoyl} amino)ethyl dihydrogen phosphate;

2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-[[2-[Bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate ;

2-[(2-Iodoethyl)-2,4-dinitro-6-({[2-(phosphonooxy)ethyl]amino}carbonyl)-anilino]ethyl methanesulfonate;

2-[(2-Chloroethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

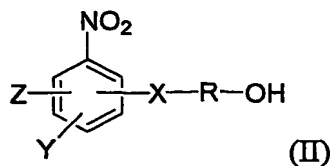
2-({3-[Bis(2-bromoethyl)amino]-2,6-dinitrobenzoyl}amino)ethyl dihydrogen phosphate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[2-(phosphonooxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate;

2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate; and

2-[(2-Iodoethyl)-2,4-dinitro-3-[[[3-(phosphonooxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate.

In a second aspect, the present invention provides alcohol compounds of Formula II



wherein:

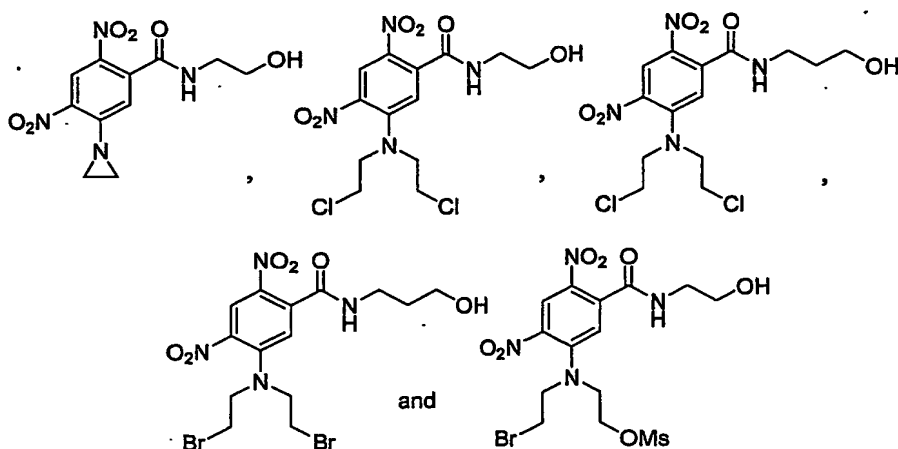
X may represent at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

Y may represent at any available ring position -N-aziridinyl, -N(CH₂CH₂W)₂, or -N(CH₂CH MeW)₂ where each W is independently selected from halogen or -OSO₂Me;

Z may represent at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

R may represent a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and

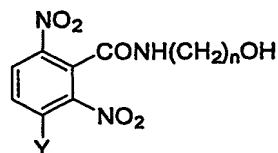
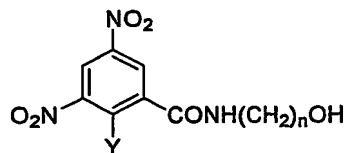
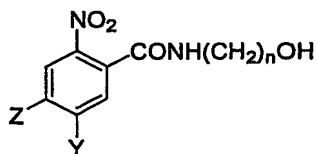
pharmaceutically acceptable salts and derivatives thereof, with the proviso that



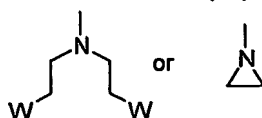
are excluded.

5

In a preferred embodiment, the alcohol compound of Formula (II) is selected from a compound represented by formulae (IIa), (IIb) or (IIc)



wherein Y may represent



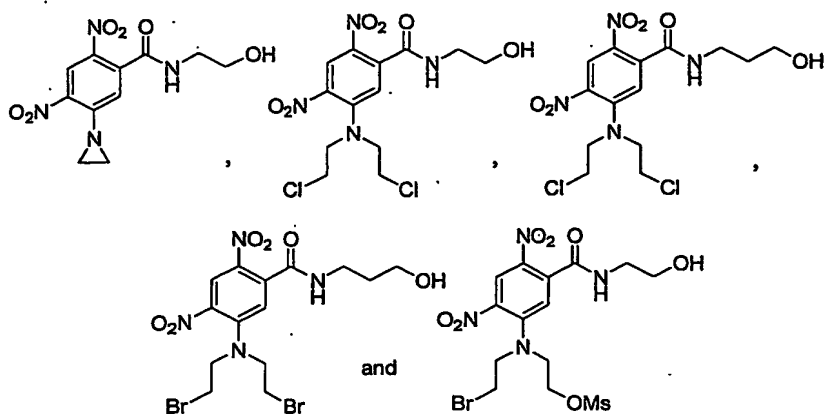
10 and wherein

n may represent 1 to 6

Z may represent $-\text{NO}_2$, -halogen, -CN, $-\text{CF}_3$ or $-\text{SO}_2\text{Me}$; and

where each W is independently selected from halogen or $-\text{OSO}_2\text{Me}$

and pharmaceutically acceptable salts and derivatives thereof with the proviso that



are excluded.

5 Preferably the compound of Formula II is selected from the following:

N-(3-Hydroxypropyl)-5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzamide;

N-(3-Hydroxypropyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

10 N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide;

5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-(methylsulfonyl)-2-nitrobenzamide;

2[(2-Bromoethyl)-5-[[3-hydroxypropyl]amino]carbonyl]-2,4-dinitroanilino]ethyl
methanesulfonate;

15 5-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2,4-dinitrobenzamide;

2-Chloro-2-[bis(2-chloroethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;

2-[Bis(2-Chloroethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;

2-[Bis(2-chloroethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;

20 2-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-3,5-dinitrobenzamide;

2-[Bis(2-chloroethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-3,5-dinitrobenzamide;

2-[Bis(2-chloroethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(5-hydroxypentyl)-3,5-dinitrobenzamide;

25 2-[Bis(2-chloroethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;

2-[Bis(2-bromoethyl)amino]-N-(6-hydroxyhexyl)-3,5-dinitrobenzamide;
 2-[Bis(2-bromopropyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;
 2-((2-Bromoethyl)-2-[[2-(2-hydroxypropyl)amino]carbonyl]-4,6-dinitroanilino)ethyl
 methanesulfonate;

5 2-((2-Bromoethyl)-2-[[2-(2-hydroxyethyl)amino]carbonyl]-4,6-dinitroanilino)ethyl
 methanesulfonate;

2-((2-Chloroethyl)-2-[[2-(2-hydroxyethyl)amino]carbonyl]-4,6-dinitroanilino)ethyl
 methanesulfonate;

2-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-3,5-dinitrobenzamide;

10 2-((2-Iodoethyl)-2-[[2-(2-hydroxyethyl)amino]carbonyl]-4,6-dinitroanilino)ethyl
 methanesulfonate;

3-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-2,6-dinitrobenzamide;

2-((2-Bromoethyl)-3-[[2-(2-hydroxyethyl)amino]carbonyl]-2,6-dinitroanilino)ethyl
 methanesulfonate;

15 3-[Bis(2-bromoethyl)amino]-N-(3-hydroxypropyl)-2,6-dinitrobenzamide;

2-((2-bromoethyl)-3-[[3-(3-hydroxypropyl)amino]carbonyl]-2,6-dinitroanilino)ethyl
 methanesulfonate;

3-[Bis(2-bromoethyl)amino]-N-(4-hydroxybutyl)-2,6-dinitrobenzamide;

2-((2-Bromoethyl)-3-[[4-(4-hydroxybutyl)amino]carbonyl]-2,6-dinitroanilino)ethyl

20 methanesulfonate;

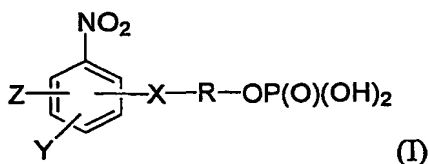
3-(3-[[3-(3-Hydroxypropyl)amino]carbonyl]{3-[(methylsulfonyl)oxy]butyl}-2,4-
 dinitroanilino)-1-methylpropyl methanesulfonate;

2-((2-Bromoethyl)-3-[[3-(3-hydroxypropyl)amino]carbonyl]-2,6-dinitroanilino)ethyl
 methanesulfonate;

25 2-((2-Chloroethyl)-3-[[3-(3-hydroxypropyl)amino]carbonyl]-2,6-dinitroanilino)ethyl
 methanesulfonate; and

2-((2-Iodoethyl)-3-[[3-(3-hydroxypropyl)amino]carbonyl]-2,6-dinitroanilino)ethyl
 methanesulfonate.

30 In a third aspect of the invention there is provided a method of preparing the phosphates
 represented by the general formula (I);



wherein:

X may represent at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or
 5 -NHSO₂-;

R may represent a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom;

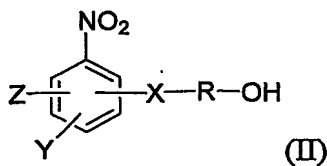
Y may represent at any available ring position -N-aziridiny1 or -N(CH₂CH₂W)₂, where each W
 10 is independently selected from halogen or -OSO₂Me;

Z may represent at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

and pharmaceutically acceptable salts and derivatives thereof;

15 the method including the step of

(i) phosphorylating a compound of formula (II)



20 wherein:

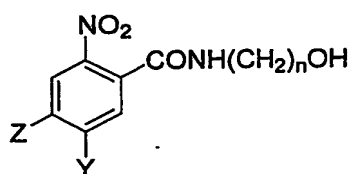
X may represent at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or
 -NHSO₂-;

Y may represent at any available ring position -N-aziridiny1, -N(CH₂CH₂W)₂, or -N(CH₂CH
 25 MeW)₂ where each W is independently selected from halogen or -OSO₂Me;

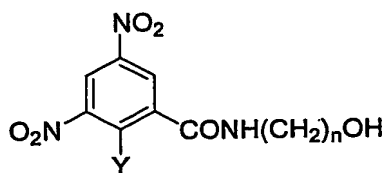
Z may represent at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

R may represent a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom.

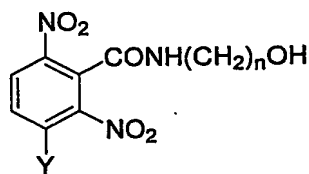
In a fourth aspect there is provided a method of preparing a compound of formulae (IIa), (IIb)
5 or (IIc)



(IIa)

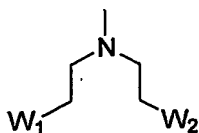


(IIb)



(IIc)

wherein Y may represent



and wherein

10 n may represent 1 to 6

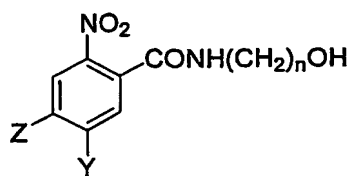
Z may represent -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

where W₁ is halogen and W₂ is -OSO₂Me

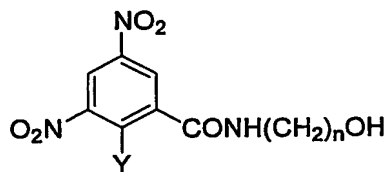
and pharmaceutically acceptable salts and derivatives thereof;

15 the method including the step of

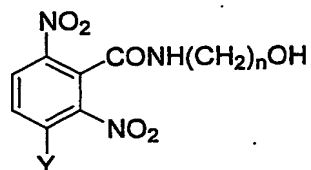
reacting a compound of formulae (IIa'), (IIb') or (IIc') optionally with heating



(IIa')

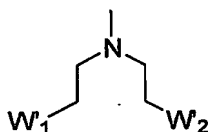


(IIb')



(IIc')

wherein Y may represent

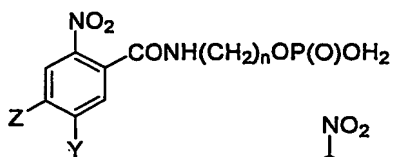


wherein W'_1 and W'_2 are each halogen;

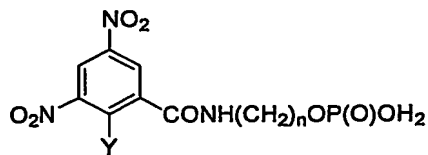
with an effective amount of silver methanesulfonate (AgOMs) in a solvent to give a compound of formulae (IIa), (IIb) or (IIc) defined above.

Preferably the solvent is selected from MeCN or other polar non-protic solvent.

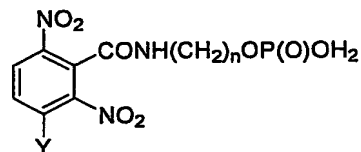
In a fifth aspect there is provided a method of preparing a compound of formulae (Ia), (Ib) or (Ic)



(Ia)

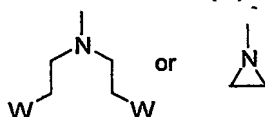


(Ib)



(Ic)

wherein Y may represent



and wherein

n may represent 1 to 6

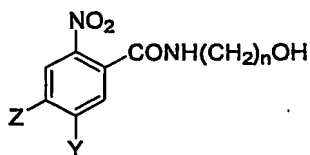
Z may represent -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

5 where each W is independently selected from halogen or -OSO₂Me

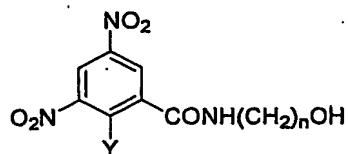
and pharmaceutically acceptable salts and derivatives thereof

the method including the step of

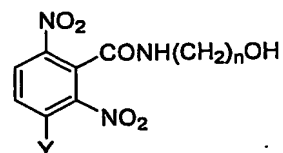
phosphorylating a compound represented by formulae (IIa), (IIb) or (IIc)



(IIa)

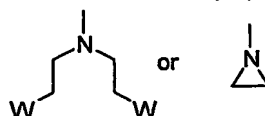


(IIb)



(IIc)

wherein Y may represent



and wherein

n may represent 1 to 6

Z may represent -NO₂, -halogen, -CN, -CF₃ or -SO₂Me; and

where each W is independently selected from halogen or -OSO₂Me

5 and pharmaceutically acceptable salts and derivatives.

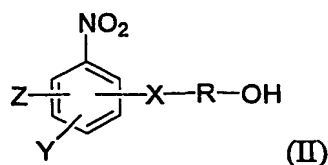
In a sixth aspect there is provided a compound of formula (I), formula (Ia), (Ib) or (Ic) or formula (IIa), (IIb) or (IIc) obtained by any one of the preparative methods defined above.

10 In a seventh aspect, the present invention provides a method for the use as prodrugs suitable for

(i) GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme; or

(ii) as one or more hypoxia-selective cytotoxins,

15 including the step of administering a compound of Formula I as defined above or a compound of Formula II



wherein:

20 X may represent at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

Y may represent at any available ring position -N-aziridinyl, -N(CH₂CH₂W)₂, or -N(CH₂CHMeW)₂ where each W is independently selected from halogen or -OSO₂Me;

25 Z may represent at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

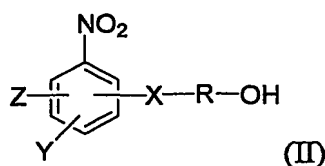
R may represent a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof;

30 or a mixture thereof in a therapeutically effective amount to tumour cells in a subject.

Preferably, the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E. coli* or by orthologous genes in *Clostridia* species.

- 5 Preferably the method includes the further step of irradiating the tumour cells.

In an eighth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as an anticancer agent including the step of administering a compound of Formula I as defined above or a compound of Formula II



wherein:

- 15 X may represent at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

Y may represent at any available ring position -N-aziridinyl, -N(CH₂CH₂W)₂ or -N(CH₂CH MeW)₂, where each W is independently selected from halogen or -OSO₂Me;

- 20 Z may represent at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

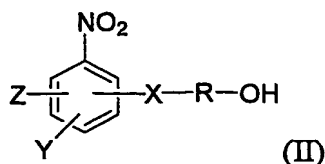
R may represent a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof;

- 25 or a mixture thereof in a therapeutically effective amount to target tumour cells in a subject.

Preferably the nitroreductase enzyme is encoded for by the *nfsB* gene of *E. coli* or by orthologous genes in *Clostridia* species.

- 30 Preferably the method includes the further step of irradiating the tumour cells.

In a ninth aspect of the present invention, there is provided a method of cell ablation therapy utilising at least one nitroreductase enzyme, wherein the method includes the step of administering a compound of Formula I as defined a above or a compound of Formula II



wherein:

X may represent at any available ring position $-\text{CONH}-$, $-\text{SO}_2\text{NH}-$, $-\text{O}-$, $-\text{CH}_2-$, $-\text{NHCO}-$ or $-\text{NHSO}_2-$;

Y may represent at any available ring position $-\text{N-aziridiny}$, $-\text{N}(\text{CH}_2\text{CH}_2\text{W})_2$ or $-\text{N}(\text{CH}_2\text{CH MeW})_2$, where each W is independently selected from halogen or $-\text{OSO}_2\text{Me}$;

Z may represent at any available ring position $-\text{NO}_2$, $-\text{halogen}$, $-\text{CN}$, $-\text{CF}_3$ or $-\text{SO}_2\text{Me}$;

R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof,

or a mixture thereof in a therapeutically effective amount to ablate tumour cells in tissue in a subject, wherein said tissue expresses the at least one nitroreductase enzyme.

Preferably the nitroreductase enzyme is encoded for by the *nfsB* gene in *E. coli* or by orthologous genes in *Clostridia* species.

Preferably, the cell ablation therapy provides a substantially minimal bystander effect.

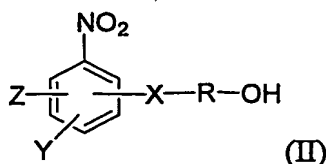
Preferably the method includes the further step of irradiating the tumour cells.

In a tenth aspect, the present invention provides a method of providing anticancer therapy, wherein a compound of Formula I as defined above is administered in a therapeutically effective amount to tumour cells in a subject.

- 5 Preferably the therapeutically effective amount of said method is between about 75% to 100% of the maximum tolerated dose of said subject.

Preferably, the method includes the further step of irradiating the tumour cells.

- 10 In an eleventh aspect of the present invention there is provided a pharmaceutical composition including a therapeutically effective amount of a compound of Formula I or a compound of Formula II



- 15 wherein:

X may represent at any available ring position $-\text{CONH}-$, $-\text{SO}_2\text{NH}-$, $-\text{O}-$, $-\text{CH}_2-$, $-\text{NHCO}-$ or $-\text{NHSO}_2-$;

- 20 Y may represent at any available ring position $-\text{N-aziridiny}$ l, $-\text{N}(\text{CH}_2\text{CH}_2\text{W})_2$ or $-\text{N}(\text{CH}_2\text{CH MeW})_2$, where each W is independently selected from halogen or $-\text{OSO}_2\text{Me}$;

Z may represent at any available ring position $-\text{NO}_2$, -halogen, $-\text{CN}$, $-\text{CF}_3$ or $-\text{SO}_2\text{Me}$;

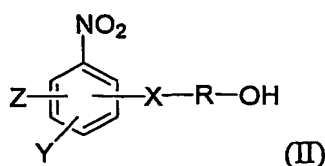
- 25 R may represent a lower C_{1-6} alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, or a mixture thereof, and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

The pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser should preferably be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, such as cutaneous, subcutaneous, or intravenous. It is to be appreciated that these factors could be readily determined by someone skilled in the art without undue experimentation.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such as gelatin.

For intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has a suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride injection, Ringer's injection, Lactated Ringer's injection. Preservatives, stabilisers, buffers antioxidants and/or other additives may be included as required.

In a twelfth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined above or a compound of Formula II



wherein:

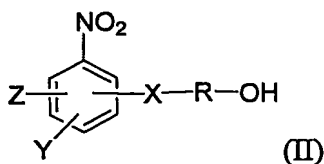
X may represent at any available ring position $-\text{CONH}-$, $-\text{SO}_2\text{NH}-$, $-\text{O}-$, $-\text{CH}_2-$, $-\text{NHCO}-$ or $-\text{NHSO}_2-$;

Y may represent at any available ring position -N-aziridinyl, -N(CH₂CH₂W)₂ or -N(CH₂CHMeW)₂, where each W is independently selected from halogen or -OSO₂Me;

Z may represent at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

R may represent a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, or mixtures thereof, for use in (i) GDEPT or (ii) as a hypoxia selective cytotoxin, to target cancer cells in a subject in need thereof.

In a thirteenth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined above or a compound of Formula II



wherein:

X may represent at any available ring position -CONH-, -SO₂NH-, -O-, -CH₂-, -NHCO- or -NHSO₂-;

Y may represent at any available ring position -N-aziridinyl, -N(CH₂CH₂W)₂ or -N(CH₂CHMeW)₂, where each W is independently selected from halogen or -OSO₂Me;

Z may represent at any available ring position -NO₂, -halogen, -CN, -CF₃ or -SO₂Me;

R may represent a lower C₁₋₆ alkyl optionally substituted with one or more groups including hydroxy, amino and N-oxides therefrom or dialkylamino and N-oxides therefrom; and pharmaceutically acceptable salts and derivatives thereof, or mixtures thereof for use in cell ablation therapy to target cancer cells in a subject in need thereof.

While the compounds of the present invention will typically be used to target tumour cells or tumour tissues in human subjects, they may be used to target tumour cells or tissues in other warm blooded animal subjects such as other primates, farm animals such as cattle, and sports animals and pets such as horses, dogs, and cats.

As used throughout the specification the term "therapeutically effective amount", is to be understood as an amount of a compound of Formula I or Formula II as defined above or a compound of any one of compounds Ia-Ic, or IIa-IIc as defined above or a mixture thereof that is sufficient to show benefit to a subject with cancer cells. The actual amount, rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment is within the responsibility of general practitioners and other medical doctors.

As used throughout the specification the term "halogen" includes chlorine, bromine or iodine.

It is to be understood that the compounds of the invention as defined above may be administered alone or in combination with other treatments, especially radiotherapy and cytotoxic chemotherapeutic drugs, either simultaneously or sequentially dependent upon the condition to be treated.

As used throughout the specification the pharmaceutically acceptable derivatives and salts thereof include acid derived salts formed from are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic acids and the like and base derived salts formed from sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine and the like.

The technique of cell ablation therapy, would be known to someone skilled in the art. This therapy can be used to selectively ablate specified target cells or tissue through specific enzymatic expression of a nitroreductase for example, that is specifically

expressed by the tissue and which can then be employed to activate a prodrug into an active metabolite to ablate the specified target cells or tissue. (Gusterson *et al.* *Endocrine Related Cancer*, 1997, 4, 67-74.)

5 The expression "substantially minimal bystander effect" is to be understood as meaning that the killing of adjoining non-targeted cells is minimal as a result of diffusion between the targeted and non-targeted cells of an activated metabolite that arises from the enzymatic activation of a compound of Formula I or Formula II as defined above or a compound of any one of compounds Ia-Ic, or IIa-IIc as defined above or a mixture thereof.

10

Pharmaceutically acceptable salts of formula (I) include the basic or acidic compounds of formula (I) that form pharmaceutically acceptable salts with both organic and inorganic acids and/or organic and inorganic bases. Examples of suitable acids for salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic, and the like. Examples of suitable bases for salt formation are sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine, and the like.

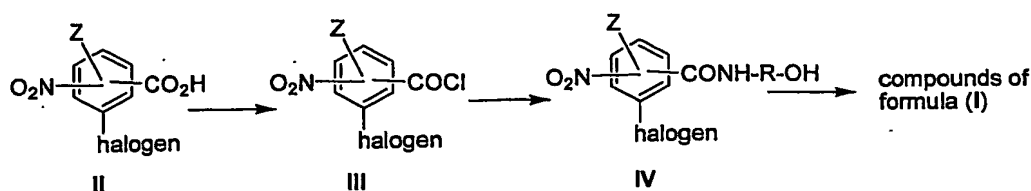
15

Further aspects of the present invention will become apparent from the following description given by way of example only and with reference to the accompanying synthetic schemes.

20

The compounds of Formula I where X is -CONH- can be prepared by the processes described in Scheme 1, where Z is as defined above for formula (I).

Scheme 1



25

The following Tables 1a and 2a set out physical data for compounds within the general Formula (I) and (II), representative of it, and capable of being prepared by the processes of the invention.

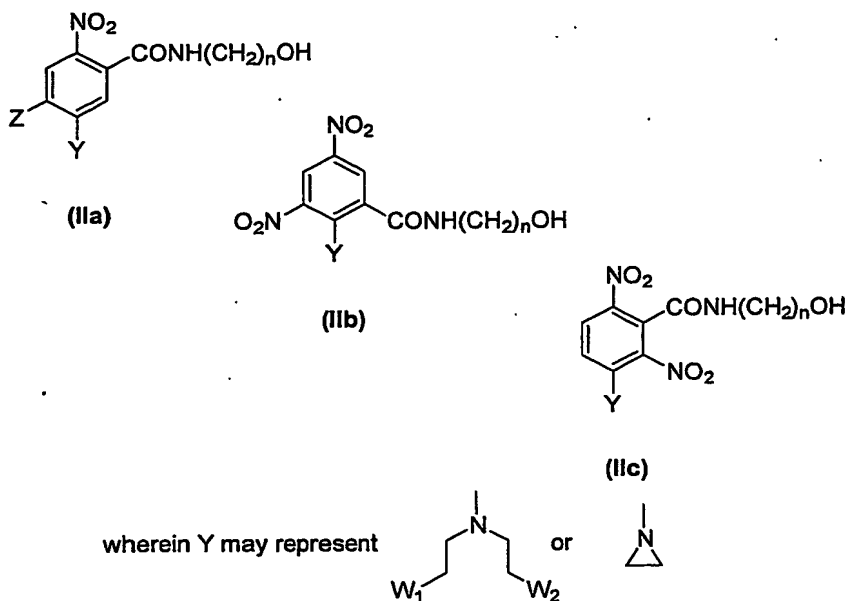


Table 1a. Representative examples of parent alcohols

| No | Z | Y | Y (W ₁ ,W ₂) | n | mp | formula or ref | analyses |
|--------|--------------------|-----------|--|---|---------|---|-------------|
| IIa-1 | NO ₂ | aziridine | - | 2 | 192-193 | Ref. 1 | C, H, N |
| IIa-2 | NO ₂ | - | Cl,Cl | 2 | | Ref. 2 | |
| IIa-3 | NO ₂ | - | Cl,Cl | 3 | 90-91 | Ref 4 | C, H, N, Cl |
| IIa-7 | NO ₂ | - | Br,Br | 2 | 151-152 | C ₁₃ H ₁₆ Br ₂ N ₄ O ₆ | C, H, N, Br |
| IIa-7s | SO ₂ Me | - | Br,Br | 2 | 126-127 | C ₁₄ H ₁₉ Br ₂ N ₃ O ₆ S | C, H, N |
| IIa-8 | NO ₂ | - | Br,Br | 3 | 85-86 | Ref 4 | C, H, N, Br |
| IIa-9 | NO ₂ | - | Br,Br | 4 | 123-124 | C ₁₅ H ₂₀ Br ₂ N ₄ O ₆ | C, H, N, Br |
| IIa-10 | NO ₂ | - | Br,Br | 5 | gum | C ₁₆ H ₂₂ Br ₂ N ₄ O ₆ | HRMS |
| IIa-11 | NO ₂ | - | Br,Br | 6 | gum | C ₁₇ H ₂₄ Br ₂ N ₄ O ₆ | HRMS |
| IIa-12 | NO ₂ | - | Br,OMs | 2 | | Ref. 2 | |
| IIa-13 | NO ₂ | - | Br,OMs | 3 | gum | C ₁₆ H ₂₁ BrN ₄ O ₉ S | HRMS |
| IIa-14 | NO ₂ | - | I,I | 2 | 142-143 | C ₁₃ H ₁₆ I ₂ N ₄ O ₆ | C, H, N, I |
| IIb-1 | - | aziridine | - | 6 | 189-192 | C ₁₅ H ₂₀ N ₄ O ₆ | C, H, N |
| IIb-2 | - | - | Cl,Cl | 2 | 109-111 | C ₁₃ H ₁₆ Cl ₂ N ₄ O ₆ | C, H, N |
| IIb-3 | - | - | Cl,Cl | 3 | 89-91 | C ₁₄ H ₁₈ Cl ₂ N ₄ O ₆ | C, H, N, Cl |

| | | | | | | | |
|--------|---|---|--------------------|---|---------|---|-------------|
| IIb-4 | - | - | Cl,Cl | 4 | gum | C ₁₅ H ₂₀ Cl ₂ N ₄ O ₆ | HRMS |
| IIb-5 | - | - | Cl,Cl | 5 | gum | C ₁₆ H ₂₂ Cl ₂ N ₄ O ₆ | HRMS |
| IIb-6 | - | - | Cl,Cl | 6 | gum | C ₁₇ H ₂₄ Cl ₂ N ₄ O ₆ | HRMS |
| IIb-2m | | | Cl,OMs | 2 | gum | C ₁₄ H ₁₉ ClN ₄ O ₉ S | HRMS |
| IIb-7 | - | - | Br,Br | 2 | 105-108 | C ₁₃ H ₁₆ Br ₂ N ₄ O ₆ | C, H, N, Br |
| IIb-7a | - | - | Br,Br ^A | 2 | 127-130 | C ₁₅ H ₂₀ Br ₂ N ₄ O ₆ | C, H, N |
| IIb-8 | - | - | Br,Br | 3 | 89-94 | C ₁₄ H ₁₈ Br ₂ N ₄ O ₆ | C, H, N, Br |
| IIb-9 | - | - | Br,Br | 4 | gum | C ₁₅ H ₂₀ Br ₂ N ₄ O ₆ | HRMS |
| IIb-10 | - | - | Br,Br | 5 | gum | C ₁₆ H ₂₂ Br ₂ N ₄ O ₆ | HRMS |
| IIb-11 | - | - | Br,Br | 6 | gum | C ₁₇ H ₂₄ Br ₂ N ₄ O ₆ | HRMS |
| IIb-12 | - | - | Br,OMs | 2 | | Ref. 3 | |
| IIb-13 | - | - | Br,OMs | 3 | gum | C ₁₅ H ₂₁ BrN ₄ O ₉ S | HRMS |
| IIb-14 | - | - | I,I | 2 | 129-131 | C ₁₃ H ₁₆ I ₂ N ₄ O ₆ | C, H, N |
| IIb-15 | - | - | I,OMs | 2 | gum | C ₁₄ H ₁₉ IN ₄ O ₉ S | HRMS |
| IIc-6 | | | Cl,OMs | 3 | 104-109 | C ₁₅ H ₂₁ ClN ₄ O ₉ S | C, H, N |
| IIc-7 | - | - | Br,Br | 2 | gum | C ₁₃ H ₁₆ Br ₂ N ₄ O ₆ | HRMS |
| IIc-8 | - | - | Br,Br | 3 | gum | C ₁₆ H ₂₂ Br ₂ N ₄ O ₆ | HRMS |
| IIc-9 | - | - | Br,Br | 4 | gum | C ₁₇ H ₂₄ Br ₂ N ₄ O ₆ | HRMS |
| IIc-12 | - | - | Br,OMs | 2 | 94-97 | C ₁₄ H ₁₉ BrN ₄ O ₉ S | C, H, N |
| IIc-13 | - | - | Br,OMs | 3 | 115-117 | Ref. 3 | C, H, N |
| IIc-14 | - | - | Br,OMs | 4 | 114-117 | C ₁₆ H ₂₃ BrN ₄ O ₉ S | C, H, N |
| IIc-15 | | | I,OMs | 3 | 100-103 | C ₁₅ H ₂₁ IN ₄ O ₉ S | C, H, N |

^Aα-methyl mustard

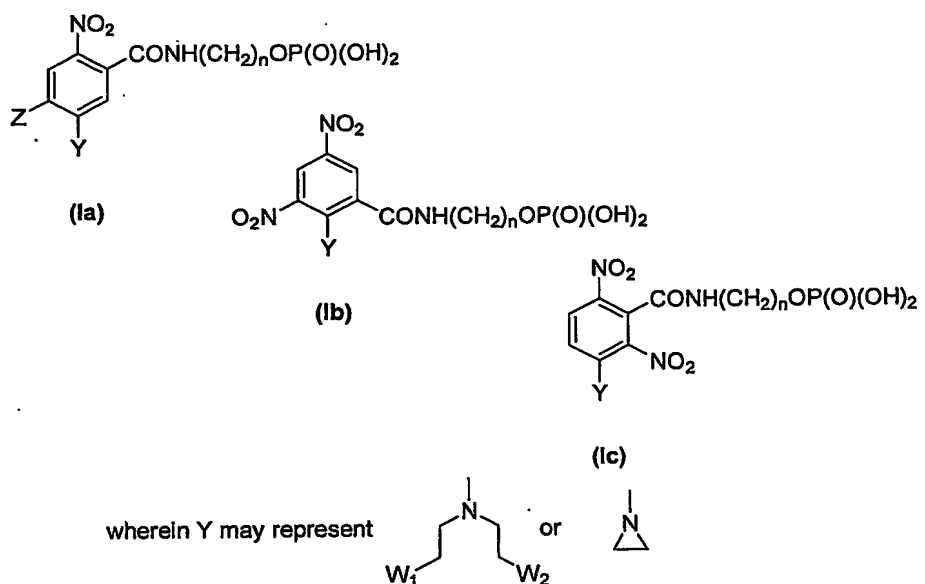
Notes

References for known compounds.

- 5 1. Khan AH, Ross WCJ. Tumor-growth inhibitory nitrophenylaziridines and related compounds. Structure-activity relations. II. Chem.-Biol. Int., 1971, 4, 11-22.
- 2 NZ Patent No.240785
3. Co-pending NZ Application No. 521851

4. Wilson WR, Pullen SM, Hogg A, Helsby NA, Hicks KO, Denny WA. Quantitation of bystander effects in nitroreductase suicide gene therapy using three-dimensional cell cultures. *Cancer Res.*, 2002, 62, 1425-1432.

- 5 The compounds of Table 1a can be prepared by the general methods set out in Schemes 2a-2k, and exemplified in Examples 1-20 below.



10

Table 1b. Examples of phosphates of formulae Ia-Ic

| No | Z | Y (W ₁ , W ₂) | n | mp | formula | analyses |
|--------|-----------------|---|---|---------|---|----------|
| Ia-3P | NO ₂ | Cl, Cl | 3 | 195-200 | C ₁₄ H ₁₉ Cl ₂ N ₄ O ₉ P | HRMS |
| Ia-8P | NO ₂ | Br, Br | 3 | 170-174 | C ₁₄ H ₁₇ Cl ₂ N ₄ O ₉ P | HRMS |
| Ib-2P | - | Cl, Cl | 2 | Foam | C ₁₃ H ₁₇ Cl ₂ N ₄ O ₉ P | HRMS |
| Ib-2mP | | Cl, OMs | 2 | 132-134 | C ₁₄ H ₂₀ ClN ₄ O ₁₂ PS | C, H, N |
| Ib-7P | - | Br, Br | 2 | Foam | C ₁₃ H ₁₇ Br ₂ N ₄ O ₉ P | HRMS |
| Ib-7aP | - | Br, Br ^A | 2 | 157-161 | C ₁₅ H ₂₁ Br ₂ N ₄ O ₉ P | C, H, N |
| Ib-12P | - | Br, OMs | 2 | Foam | C ₁₄ H ₂₁ BrN ₄ O ₁₂ PS | HRMS |
| Ib-14P | - | I, I | 2 | Foam | C ₁₃ H ₁₇ I ₂ N ₄ O ₉ P | HRMS |

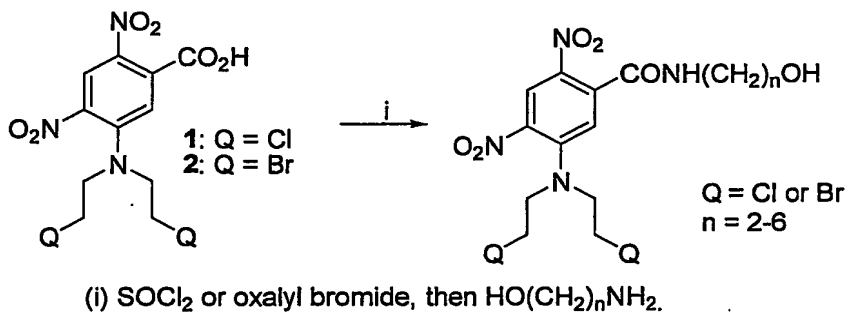
| | | | | | | |
|---------------|---|--------|---|---------|---|---------|
| Ib-15P | - | I,OMs | 2 | 147-150 | C ₁₄ H ₂₀ IN ₄ O ₁₂ PS | C, H, N |
| Ic-6P | | Cl,OMs | 3 | 88-92 | C ₁₅ H ₂₂ ClN ₄ O ₁₂ PS | C, H, N |
| Ic-8P | - | Br,Br | 3 | Foam | C ₁₄ H ₁₉ Br ₂ N ₄ O ₉ P | HRMS |
| Ic-12P | | Br,OMs | 2 | 93-97 | C ₁₄ H ₂₀ BrN ₄ O ₁₂ PS | C, H, N |
| Ic-13P | - | Br,OMs | 3 | foam | C ₁₅ H ₂₂ BrN ₄ O ₁₂ PS | HRMS |
| Ic-15P | | I,OMs | 3 | | C ₁₅ H ₂₂ IN ₄ O ₁₂ PS | |

^Aalpha-Me

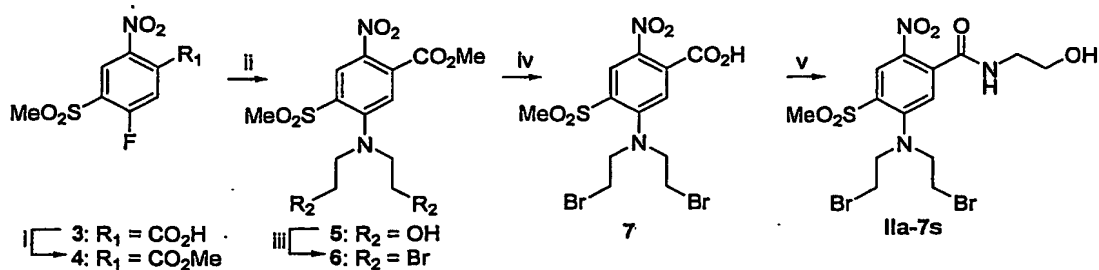
The compounds of Table 1b can be prepared by the general methods set out in Scheme 3, and exemplified in Examples 26-39 below.

5

Scheme 2a

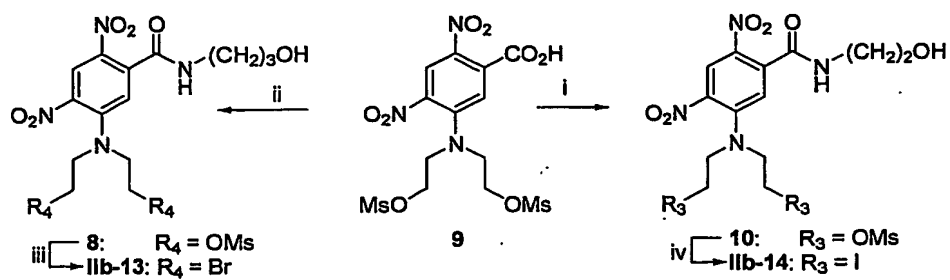


Scheme 2b

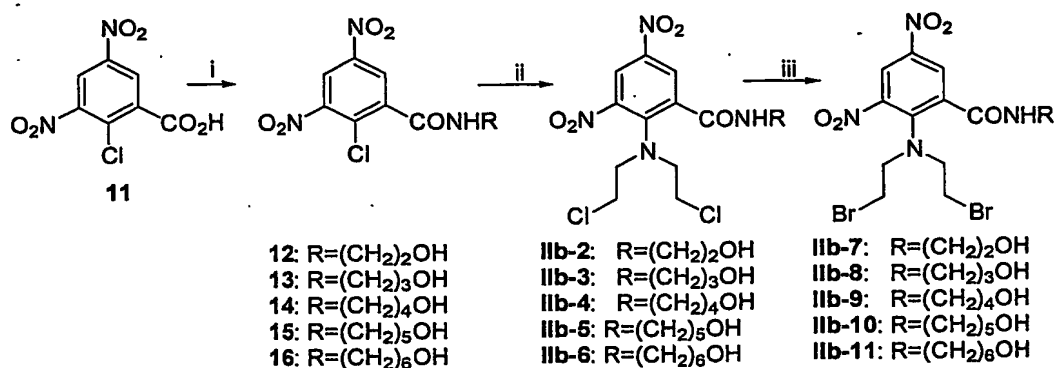


(i) SOCl₂/MeOH;
(ii) diethanolamine;
(iii) MsCl/py, then LiBr;
(iv) KOH;
(v) (COCl)₂/cat. DMF, then 2-aminoethanol

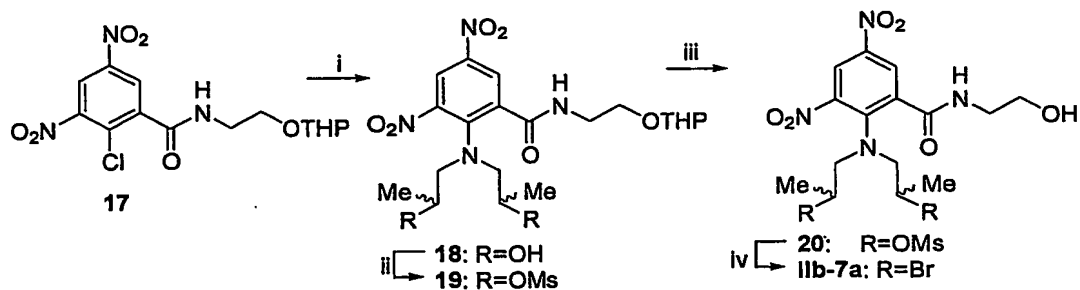
10

Scheme 2c

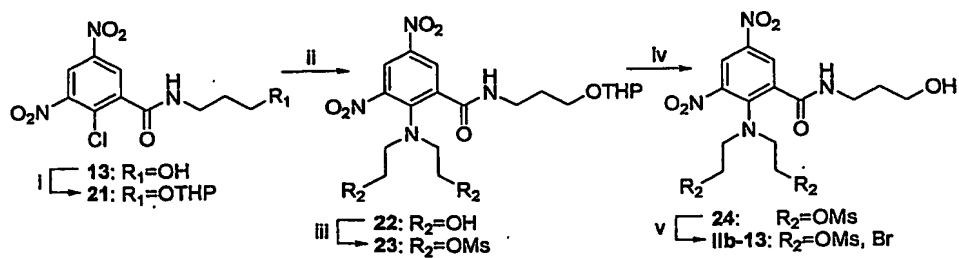
- (i) $\text{SOCl}_2/\text{cat DMF}$ or $(\text{COCl})_2/\text{cat DMF}$, then 2-aminoethanol
 (ii) $\text{SOCl}_2/\text{cat DMF}$ or $(\text{COBr})_2/\text{cat DMF}$, then 3-amino-1-propanol
 (iii) LiBr (1.4 equiv.)/DMF;
 (iv) NaI (excess)/MeCN.

Scheme 2d

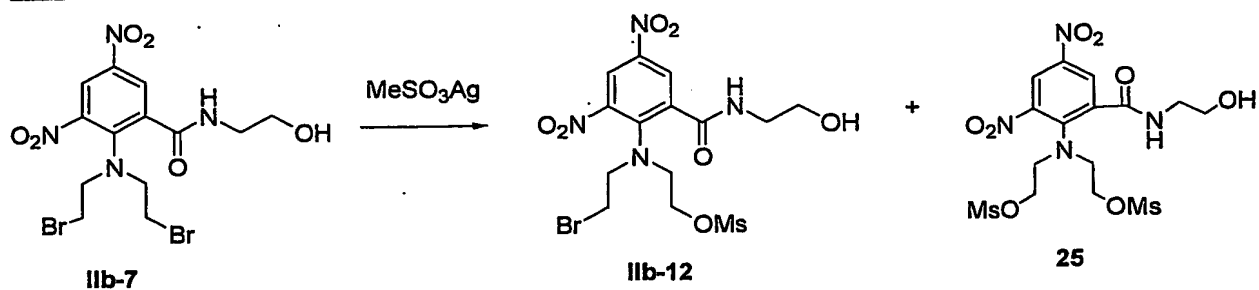
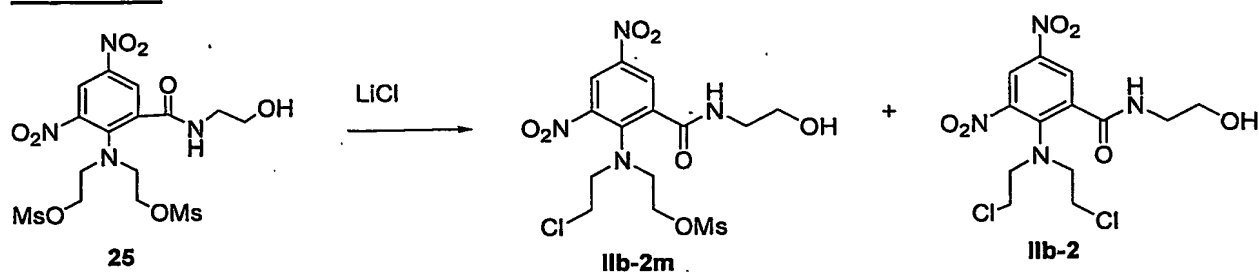
- (i) SOCl₂/cat. DMF, then RNH₂;
(ii) HN(CH₂CH₂Cl)₂;
(iii) LiBr/3-Mebutanone

Scheme 2e

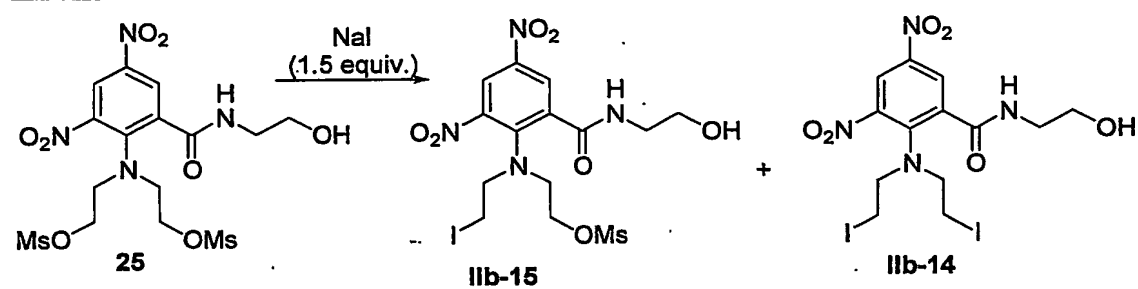
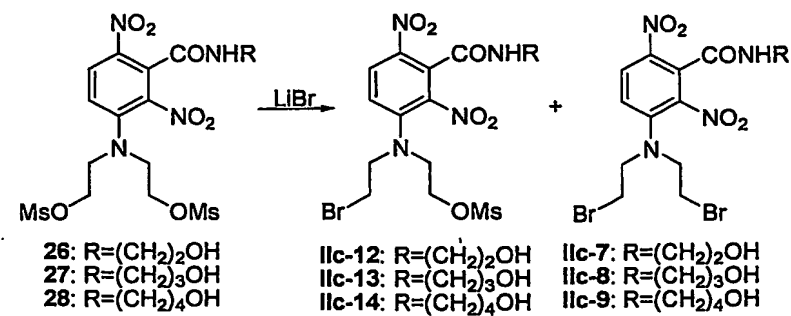
- (i) diisopropanolamine;
(ii) MsCl/Et₃N;
(iii) 1N HCl/THF;
(iv) LiBr (excess).

Scheme 2f

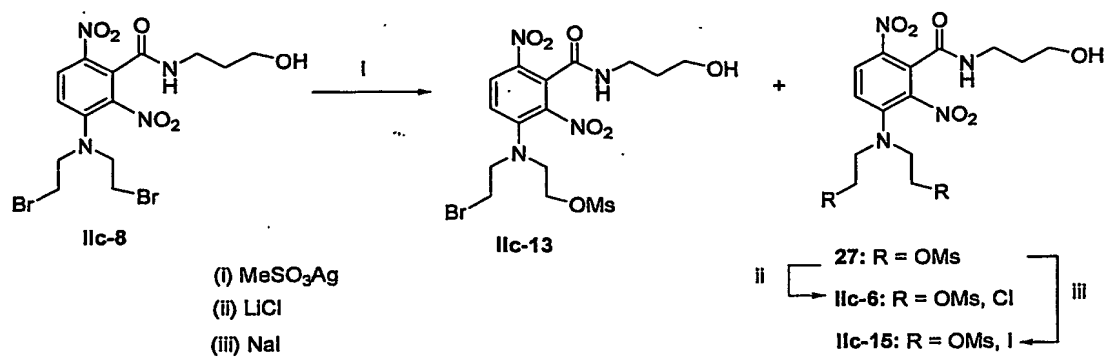
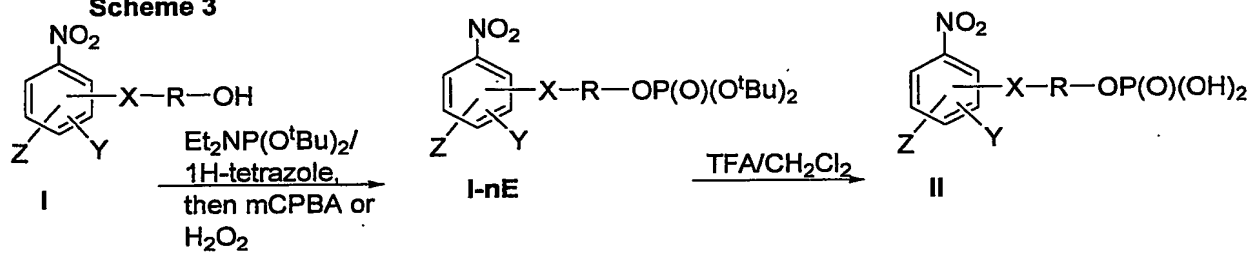
- (i) 3,4-dihydro-2H-pyran, *p*-TsOH;
(ii) diethanolamine;
(iii) MsCl/Et₃N;
(iv) 1N HCl/THF;
(v) LiBr (1.2eq).

Scheme 2g**Scheme 2h**

5

Scheme 2i**Scheme 2j**

10

Scheme 2k**Scheme 3**

In Scheme 3, X, Y, Z, and R are as specified for formula (I) and (II) above.

Examples

The invention and the best mode for practising the same are illustrated by the following Examples 1-25 (alcohols) and Examples 26-39 (phosphates).

5

Example 1 (Scheme 2a). N-(3-Hydroxypropyl)-5-[bis(2-chloroethyl)amino]-2,4-

dinitrobenzamide (IIa-3). A suspension of 5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzoic acid [Palmer et al., J. Med. Chem., 1994, 37, 2175] (1) (2.50 g, 7.1 mmol) in SOCl₂ (20 mL) containing DMF (2 drops) was heated under reflux for 1 h, then concentrated to dryness under reduced pressure and re-evaporated with benzene. The resulting crude benzoyl chloride was dissolved in Me₂CO (50 mL) and the cooled (-5 °C) solution was treated with a cold solution of 3-amino-1-propanol (1.09 g, 14.5 mmol) in water (25 mL). The reaction mixture was shaken at room temperature for 5 min, then diluted with water (25 mL), concentrated to half volume, and extracted with CH₂Cl₂ (2x). The organic extract was washed with 0.1 N HCl and water then worked up to give a solid which was chromatographed on silica gel, eluting with EtOAc to give **IIa-3** (2.37 g, 82%): mp (EtOAc/i-Pr₂O) 90-91 °C; ¹H NMR [(CD₃)₂SO] δ 8.63 (t, *J* = 5.6 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.42 (s, 1 H, H-6), 4.46 (t, *J* = 5.1 Hz, 1 H, OH), 3.82 (t, *J* = 5.9 Hz, 4 H, N(CH₂CH₂Cl)₂), 3.68 (t, *J* = 5.9 Hz, 4 H, N(CH₂CH₂Cl)₂), **3.49** (q, *J* = 6.0 Hz, 2 H, CH₂OH), 3.29 (q, partially obscured, *J* = 5.9 Hz, 2 H, CONHCH₂), 1.68 (pent, *J* = 6.7 Hz, 2 H, CH₂CH₂CH₂). Anal. (C₁₄H₁₈Cl₂N₄O₆) C, H, N, Cl.

20

Example 2 (Scheme 2a). N-(3-Hydroxypropyl)-5-[bis(2-bromoethyl)amino]-2,4-

dinitrobenzamide (IIa-8). A suspension of powdered 5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzoic acid (2) (1.10 g, 2.49 mmol) in benzene (170 mL) was treated at 20 °C with oxalyl bromide (1.10 mL, 11.7 mmol) and DMF (2 drops). The mixture was stirred at 20 °C for 2 h, then concentrated under reduced pressure, and re-evaporated to dryness in the presence of benzene under high vacuum. The resulting acid bromide was dissolved in Me₂CO (20 mL) and the solution was treated at -5 °C with a cold solution of 3-amino-1-propanol (0.39 g, 5.19 mmol) in water (10 mL). The mixture was shaken at room temperature for 5 min, then diluted with water and extracted with EtOAc (2x). The organic extract was worked up and the resulting residue was chromatographed on silica gel, eluting with EtOAc, to give **IIa-8** (1.06 g, 85%): mp (EtOAc/i-Pr₂O) 85-86 °C; ¹H NMR [(CD₃)₂SO] δ 8.64 (t, *J* = 5.6 Hz,

30

1 H, CONH), 8.53 (s, 1 H, H-3), 7.41 (s, 1 H, H-6), 3.77-3.64 (m, 8 H, N(CH₂CH₂Cl)₂), 4.46 (br s, 1 H, OH), 3.49 (t, *J* = 6.3 Hz, 2 H, CH₂OH), 3.33-3.25 (m, partially obscured, 2 H, CONHCH₂), 1.68 (pent, *J* = 6.72 Hz, 2 H, CH₂CH₂CH₂). Anal. (C₁₄H₁₈Br₂N₄O₆) C, H, N, Br.

- 5 **Example 3 (Scheme 2a).** N-(2-Hydroxyethyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa-7). Similar reaction of the acid bromide of 2 with 2-aminoethanol gave IIa-7 (0.78 g, 46%): mp (MeOH/EtOAc/pet. ether) 151-152 °C; ¹H NMR [(CD₃)₂SO] δ 8.73 (t, *J* = 5.7 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.43 (s, 1 H, H-6), 4.76 (t, *J* = 5.6 Hz, 1 H, OH), 3.77-3.64 (m, 8 H, N(CH₂CH₂Br)₂), 3.53 (q, *J* = 6.0 Hz, 2 H, CH₂OH), 3.31 (q, partially obscured, *J* = 6.1 Hz, 2 H, CONHCH₂). Anal. (C₁₃H₁₆Br₂N₄O₆) C, H, N, Br.

- Example 4 (Scheme 2a).** N-(4-Hydroxybutyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa-9). Similar reaction of the acid bromide of 2 with 4-amino-1-butanol in cold Me₂CO, followed by chromatography on silica gel and elution with EtOAc gave IIa-9 (69%) as a yellow solid: mp (EtOAc/iPr₂O) 123-124 °C; ¹H NMR [(CD₃)₂SO] δ 8.62 (t, *J* = 5.6 Hz, 1 H), 8.53 (s, 1 H), 7.39 (s, 1 H), 4.39 (t, *J* = 5.1 Hz, 1 H), 3.78-3.64 (m, 8 H), 3.47-3.40 (m, 2 H), 3.27-3.20 (m, 2 H), 1.61-1.44 (m, 4 H). Anal. (C₁₅H₂₀Br₂N₄O₆) C, H, N, Br.

- Example 5 (Scheme 2a).** N-(5-Hydroxypentyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa-10). Similar reaction of the acid bromide of 2 with 5-amino-1-pentanol in cold Me₂CO, followed by chromatography on silica gel and elution with EtOAc gave IIa-10 (66%) as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.62 (t, *J* = 5.6 Hz, 1 H), 8.53 (s, 1 H), 7.38 (s, 1 H), 4.34 (t, *J* = 5.1 Hz, 1 H), 3.79-3.64 (m, 8 H), 3.44-3.37 (m, 2 H), 3.26-3.18 (m, 2 H), 1.59-1.29 (m, 4 H). HRMS (FAB) Calcd. for C₁₆H₂₃⁷⁹Br₂N₄O₆ [M+H⁺] *m/z* 524.9984, found 524.9964.

- Example 6 (Scheme 2a).** N-(6-Hydroxyhexyl)-5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzamide (IIa-11). Similar reaction of the acid bromide of 2 with 6-amino-1-hexanol in cold Me₂CO, followed by chromatography on silica gel and elution with EtOAc gave IIa-11 (72%) as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.61 (t, *J* = 5.6 Hz, 1 H), 8.53 (s, 1 H), 7.38 (s, 1 H), 4.31 (t, *J* = 5.2 Hz, 1 H), 3.79-3.64 (m, 8 H), 3.43-3.36 (m, 2 H), 3.27-3.19

(m, 2 H), 1.58-1.26 (m, 4 H). HRMS (FAB) Calcd. for $C_{17}H_{25}^{79}Br_2N_4O_6$ $[M+H]^+$ m/z 539.0141, found 539.0137.

Example 7. (Scheme 2b). 5-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-4-

- 5 **(methylsulfonyl)-2-nitrobenzamide (IIa -7s).** 5-Fluoro-4-(methylsulfonyl)-2-nitrobenzoic acid [Atwell et al., ACDD, 1996, 11, 553] (3) was heated in excess $SOCl_2$ /catalytic DMF to provide the acid chloride, which was reacted with dry MeOH to give methyl 5-fluoro-4-(methylsulfonyl)-2-nitrobenzoate (4): mp (EtOAc/hexane) 134-135 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.49 (d, $J = 5.9$ Hz, 1 H), 8.14 (d, $J = 9.3$ Hz, 1 H), 3.92 (s, 3 H), 3.46 (s, 3 H). Anal. (C₉H₈FNO₆S) C, H, N.

- A mixture of 4 (1.48 g, 5.34 mmol) and diethanolamine (1.40 g, 13.3 mmol) in DMA (6 mL) was stirred at 30 °C for 1 h, and then diluted with EtOAc (60 mL). The solution was washed with brine (2x) and concentrated under reduced pressure. The residue was purified by
15 chromatography on silica gel, eluting with EtOAc/MeOH, followed by recrystallization from EtOAc/ iPr_2O , to give methyl 5-[bis(2-hydroxyethyl)amino]-4-(methylsulfonyl)-2-nitrobenzoate (5) (1.41 g, 73%): mp 99-100 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.56 (s, 1 H), 7.73 (s, 1 H), 4.62 (t, $J = 4.9$ Hz, 2 H), 3.89 (s, 3 H), 3.59-3.49 (m, 8 H), 3.45 (s, 3 H). Anal. (C₁₃H₁₈N₂O₈S) C, H, N.

- 20 A solution of 5 (1.48 g, 4.08 mmol) in dry pyridine (15 mL) was treated dropwise at 0 °C with MsCl (0.80 mL, 10.3 mmol). The reaction was stirred at 0 °C for 2 h, then poured into 10% aqueous NaBr. The resulting crude dimesylate was collected, washed well with water, dried, dissolved in DMF (15 mL) and stirred with NaBr (21.6 g, 25 mmol) at 70 °C for 1.5 h. The
25 cooled mixture was poured into water and the resulting solid was purified by chromatography, on silica gel, eluting with CH_2Cl_2 , then recrystallisation from CH_2Cl_2 / iPr_2O to give methyl 5-[bis(2-bromoethyl)amino]-4-(methylsulfonyl)-2-nitrobenzoate (6) (1.47 g, 74%): mp 161-162 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.58 (s, 1 H), 7.94 (s, 1 H), 3.90 (s, 3 H), 3.82 (t, $J = 7.0$ Hz, 4 H), 3.63 (t, $J = 6.9$ Hz, 4 H), 3.48 (s, 3 H). Anal. (C₁₃H₁₆Br₂N₂O₆S) C, H, N.

- 30 A solution of 6 (1.00 g, 2.05 mmol) in a dioxane/MeOH (1:1, 20 mL) was treated at 10 °C with 4N aqueous KOH (5 mL), and stirred at 10 °C for 45 min. The mixture was acidified to

pH 2 with 1 N aqueous HBr, concentrated to a small volume under reduced pressure, and then diluted with saturated aqueous NaBr (20 mL). The resulting semi-solid was isolated and crystallized twice from MeOH/H₂O to give 5-[bis(2-bromoethyl)amino]-4-(methylsulfonyl)-2-nitrobenzoic acid (**7**) (0.70 g, 72%): mp 174-176 °C; ¹H NMR [(CD₃)₂SO] δ 8.50 (s, 1 H), 7.88 (s, 1 H), 3.79 (t, *J* = 7.0 Hz, 4 H), 3.62 (t, *J* = 7.0 Hz, 4 H), 3.48 (s, 3 H). Anal. (C₁₂H₁₄Br₂N₂O₆S) C, H, N.

A finely-divided suspension of **7** (260 mmg, 0.55 mmol) in dry benzene (50 mL) was treated with (COBr)₂ (2.13 mL, 0.20 mmol) and catalytic DMF. The mixture was stirred for 2 h, then concentrated to dryness under reduced pressure and re-evaporated with benzene under high vacuum. The resulting crude acid bromide was dissolved in Me₂CO (10 mL) and treated at -5 °C with a cold solution of 2-aminoethanol (101 mg, 1.65 mmol) in water (5 mL). The mixture was stirred at 0 °C for 5 min, then acidified to pH 4 with 1 N aqueous HBr, and concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with EtOAc, to give **IIa-7s** (222 mg, 78%): mp (EtOAc/iPr₂O) 126-127 °C; ¹H NMR [(CD₃)₂SO] δ 8.75 (t, *J* = 5.6 Hz, 1 H), 8.51 (s, 1 H), 7.68 (s, 1 H), 4.79 (t, *J* = 5.4 Hz, 1 H), 3.76 (t, *J* = 7.1 Hz, 4 H), 3.62 (t, *J* = 7.0 Hz, 4 H), 3.54 (q, *J* = 5.9 Hz, 2 H), 3.48 (s, 3 H), 3.31 (after D₂O exchange, t, *J* = 6.0 Hz, 2 H). HRMS (FAB) calcd. for C₁₄H₂₀⁷⁹Br₂N₃O₆S (MH⁺) *m/z* 515.9440; found 515.9425.

Example 8 (Scheme 2c). 2[(2-Bromoethyl)-5-[(3-hydroxypropyl)amino]carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate (**IIb-13**) and 5-[bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-2, 4-dinitrobenzamide (**IIb-14**). 5-(Bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoic acid [A method of preparing this compound is disclosed in co-pending NZ Application No. 521851] (**9**) was heated under reflux in excess SOCl₂ (60 mL) and catalytic DMF for 1 h. Evaporation under reduced pressure, followed by azeotroping in benzene, gave the crude acid chloride. This was dissolved in dry Me₂CO and treated at 0 °C with 3-amino-1-propanol at 0 °C for 5 min. The mixture was acidified to pH 2-3 with 0.2 N HCl, concentrated to half volume, and then solid NaBr was added, followed by extraction with EtOAc (2x). Evaporation, and chromatography of the residue on silica gel, eluting with EtOAc/MeOH (9:1), gave 2-(5-[(3-hydroxypropyl)amino]carbonyl){2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl

methanesulfonate (**8**) (68%) as a yellow gum; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.54 (t, $J = 5.7$ Hz, 1 H), 8.53 (s, 1 H), 7.45 (s, 1 H), 4.43 (t, $J = 5.1$ Hz, 1 H), 4.33 (t, $J = 5.2$ Hz, 4 H), 3.69 (t, $J = 5.2$ Hz, 4 H), 3.57 (q, $J = 5.9$ Hz, 2 H), 3.26 (after D_2O exchange, t, $J = 7.0$ Hz, 1 H), 3.12 (s, 6 H), 1.66 (pent, $J = 6.7$ Hz, 2 H). HRMS (FAB) calcd. for $\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_{12}\text{S}$ (MH^+) m/z 529.0910; found 529.0904.

A solution of **8** in DMF was treated with LiBr (1.4 equiv.), and worked up as above, and the product was chromatographed on silica gel. Elution with EtOAc gave a small amount of the dibromo mustard, while elution with EtOAc/MeOH (19:1) gave **IIb-13** (31%) as a yellow gum: ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.60 (t, $J = 5.6$ Hz, 1 H), 8.54 (s, 1 H), 7.44 (s, 1 H), 4.45 (t, $J = 5.2$ Hz, 1 H), 4.33 (t, $J = 5.1$ Hz, 2 H), 3.74 (t, $J = 5.2$ Hz, 2 H), 3.72-3.66 (m, 4 H), 3.49 (q, $J = 5.9$ Hz, 2 H), 3.27 (after D_2O exchange, t, $J = 7.0$ Hz, 2 H), 3.14 (s, 3 H), 1.68 (pent, $J = 6.7$ Hz, 2 H). HRMS (FAB) calcd. for $\text{C}_{15}\text{H}_{22}^{79}\text{BrN}_4\text{O}_9\text{S}$ (MH^+) m/z 515.0270; found 515.0283.

Similar treatment of the acid chloride of **9** (activation with $(\text{COCl})_2/\text{DMF}$) with 2-aminoethanol gave 2-(5-[(2-hydroxyethyl)amino]carbonyl){2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilinoethyl methanesulfonate (**10**). A stirred mixture of **10** (1.42 g, 2.76 mmol) and NaI (3.3 g, 22 mmol) in dry MeCN (45 mL) was heated at reflux for 1 h, then concentrated under reduced pressure. The residue was partitioned between EtOAc and water, and the organic layer was washed with water and evaporated. The residue was chromatographed on silica gel, eluting with $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (1:4), followed by recrystallisation from MeOH/EtOAc/ i -Pr $_2$ O to give **IIa-14** (2.9 g, 81%): mp 142-143 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.73 (t, $J = 5.7$ Hz, 1 H), 8.53 (s, 1 H), 7.38 (s, 1 H), 4.76 (t, $J = 5.5$ Hz, 1 H), 3.68 (t, $J = 6.9$ Hz, 4 H), 3.57-3.49 (m, 2 H), 3.39 (t, $J = 6.9$ Hz, 4 H), 3.34-3.26 (m, partially obscured, 2 H). Anal. ($\text{C}_{13}\text{H}_{16}\text{I}_2\text{N}_4\text{O}_6$) C, H, N.

Example 9. 2-Chloro-2-[bis(2-chloroethyl)amino]-*N*-(6-hydroxyhexyl)-3,5-

dinitrobenzamide (**IIa-1**). A solution of 2-chloro-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (**14**) [for preparation see Example 14 below] (118 mg, 0.34 mmol) and Et_3N (200 mg) in EtOAc (200 mL) was treated with aziridine (100 mg) at room temperature for 3 h. The mixture was diluted with EtOAc and washed three times with water, after dry, concentrated under reduced pressure until about 20 mL, the yellow solid was collected and gave 101 mg

product (84%); ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, $J = 2.8$ Hz, 1 H), 8.63 (m, 1 H), 8.29 (d, $J = 2.8$ Hz, 1 H), 4.31 (m, 1 H), 3.39 (m, 2 H), 3.25 (m, 2 H), 2.37 (s, 4 H), 1.56 (m, 2 H), 1.43 (m, 2 H), 1.33 (m, 4 H). Anal. ($\text{C}_{15}\text{H}_{20}\text{N}_4\text{O}_6$) C, H, N.

Example 10 (Scheme 2d). 2-[Bis(2-Chloroethyl)amino]-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-2) and 2-[bis(2-bromoethyl)amino]-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-7).

2-Chloro-3,5-dinitrobenzoic acid (**11**) (18 g, 81 mmol) was treated with SOCl_2 (200 ml) containing one drop of DMF and heated under reflux for 2 h.

Evaporation of reagent followed by azeotroping with benzene gave the crude acid chloride, which was dissolved in Me_2CO (120 mL), cooled in ice-bath and treated with 2-aminoethanol (7.9 g). After stirring for 20 min. the reaction mixture was acidified to pH 4-5 with 1 N HCl, most of the solvent was evaporated, and the residue was partitioned between water (50 mL) and EtOAc (100 mL). The aqueous phase was extracted with EtOAc, and the combined organic phases were washed with sat. NaHCO_3 , 1 N HCl and brine respectively, then

concentrated to give 2-chloro-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (**12**) 21.34 g (91%): mp (EtOAc) 159-160 $^\circ\text{C}$; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.99 (d, $J = 2.6$ Hz, 1 H, H-4), 8.86 (m, 1 H, CONH), 8.56 (d, $J = 2.6$ Hz, 1 H, H-6), 4.83 (m, 1 H, OH), 3.54 (m, 4 H). Anal. ($\text{C}_9\text{H}_8\text{ClN}_3\text{O}_6$) C, H, N.

A solution of **12** (1.52 g, 5.3 mmol) and Et_3N (4 mL) in *p*-dioxane (60 mL) was treated with *N,N*-bis(2-chloroethyl)amine hydrochloride (3.0 g, 16.5 mmol) at 50 $^\circ\text{C}$ for 24 h. The mixture was poured into water and extracted with EtOAc to give the crude product, which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) and concentration of the eluate under reduced pressure gave a oily residue that was dissolved in minimum amount of EtOAc. Petroleum ether was added slowly until incipient cloudiness, and the solution was stood overnight to precipitate 2-chloro-2-[bis(2-chloroethyl)amino]-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (**IIb-2**) (2.07 g, 100%) as yellow crystals: mp (EtOAc/petroleum ether) 109-111 $^\circ\text{C}$; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.73 (d, $J = 2.6$ Hz, 1 H, H-4), 8.72 (m, 1 H, CONH), 8.34 (d, $J = 2.6$ Hz, 1 H, H-6), 4.83 (m, 1 H, OH), 3.72 (m, 4 H, $2 \times \text{CH}_2\text{Cl}$), 3.55 (m, 2 H), 3.42 (m, 4 H, $2 \times \text{CH}_2\text{N}$), 3.34 (m, 2 H); ^{13}C NMR δ 165.3, 145.8, 145.3, 141.0, 136.3, 127.5, 122.1, 59.1, 54.1, 42.1, 41.5. HRMS (FAB) $[\text{MH}^+]$ Calcd. For $\text{C}_{13}\text{H}_{17}^{35}\text{Cl}_2\text{N}_4\text{O}_6$ m/z 395.0525. Found; 395.0525.

A solution of **IIb-2** (1.20 g, 3.04 mmol) and LiBr (5 g) in 3-methyl-2-butanone (20 mL) was heated under reflux for 6 h, then cooled and poured into water. Extraction with EtOAc gave a crude product (<95 % pure), that was re-treated with LiBr (5 g) in 3-methyl-2-butanone for a further 4 h, then worked up and chromatographed on silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 1:0), to give **IIb-7** (1.39 g, 95%): mp (EtOAc/petroleum ether) 105-108 °C; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.7 Hz, 1 H, H-4), 8.73 (m, 1 H, CONH), 8.34 (d, *J* = 2.7 Hz, 1 H, H-6), 4.83 (m, 1 H, OH), 3.59-3.29 (m, 12 H); ¹³C NMR δ 165.3, 145.4, 145.3, 141.1, 136.5, 127.4, 122.1, 59.3, 53.9, 42.1, 30.0. HRMS (FAB) Calcd. For C₁₃H₁₇⁷⁹Br₂N₄O₆ [M+H⁺] *m/z* 482.9515. Found; 482.9492. Anal. (C₁₃H₁₆Br₂N₄O₆) H, N, Br; C: found, 32.9; calculated 32.3%.

Example 11 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-*N*-(3-hydroxypropyl)-3,5-dinitrobenzamide (IIb-3**) and 2-[bis(2-bromoethyl)amino]-*N*-(3-hydroxypropyl)-3,5-**

dinitrobenzamide (IIb-8**).** Reaction of the acid chloride of **11** (17 g) with 3-aminopropanol (7.5 g) in Me₂CO (120 mL) at 0 °C as described above, gave 2-chloro-*N*-(3-hydroxypropyl)-3,5-dinitrobenzamide (**13**) (5.06 g, 26%): mp (EtOAc/petroleum ether) 120-121 °C; ¹H NMR [(CD₃)₂SO] δ 8.99 (d, *J* = 2.6 Hz, 1 H, H-4), 8.79 (m, 1 H, CONH), 8.51 (d, *J* = 2.6 Hz, 1 H, H-6), 4.50 (m, 1 H, OH), 3.49 (m, 2 H), 3.32 (m, 2 H), 1.70 (m, 2 H). Anal. (C₁₀H₁₀ClN₃O₆) C, H, N.

A solution of **13** (1.39 g, 4.58 mmol) and Et₃N (4 mL) in *p*-dioxane (60 mL) was treated with *N,N*-bis(2-chloroethyl)amine hydrochloride (2.9 g, 16.0 mmol) at 50 °C for 24 h. Workup as described above gave **IIb-3** (1.84 g, 100%): mp (EtOAc/petroleum ether) 89-91 °C; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.7 Hz, 1 H, H-4), 8.71 (m, 1 H, CONH), 8.30 (d, *J* = 2.7 Hz, 1 H, H-6), 4.52 (m, 1 H, OH), 3.71 (m, 4 H, 2xCH₂Cl), 3.50 (m, 2 H), 3.42 (m, 4 H, 2xCH₂N), 3.32 (m, 2 H), 1.71 (m, 2 H); ¹³C NMR δ 165.1, 145.7, 145.5, 141.0, 136.4, 127.3, 122.1, 58.4, 54.1, 41.5, 36.7, 31.8. HRMS (FAB) Calcd. For C₁₄H₁₉³⁵Cl₂N₄O₆ [M+H⁺] *m/z* 409.0682. Found; 409.0678.

Treatment of **IIb-3** with LiBr in 3-methyl-2-butanone twice, as described above, gave **IIb-8** (74% yield): mp (EtOAc/petroleum ether) 89-94 °C; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.7

Hz, 1 H, H-4), 8.72 (m, 1 H, CONH), 8.30 (d, $J = 2.7$ Hz, 1H, H-6), 3.77-3.44 (m, 12 H), 1.70 (m, 2 H); ^{13}C NMR δ 165.1, 145.5, 145.3, 141.2, 136.5, 127.3, 122.1, 58.4, 54.0, 36.7, 31.8, 29.9. HRMS (FAB) Calcd. For $\text{C}_{14}\text{H}_{19}^{79}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 496.9671. Found; 496.9658.

- 5 **Example 12 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-*N*-(4-hydroxybutyl)-3,5-dinitrobenzamide (IIb-4) and 2-[bis(2-bromoethyl)amino]-*N*-(4-hydroxybutyl)-3,5-dinitrobenzamide (IIb-9).** Reaction of the acid chloride of **11** (2.65 g, 10 mmol) with 4-aminobutanol (1.9 g) as above, followed by acidification to pH 4-5 with 1 N HCl and evaporation of most of the solvent gave a residue. This was partitioned between water (50 mL) and EtOAc (100 mL). The aqueous phase was extracted with EtOAc, and the combined organic phase were washed with sat. NaHCO_3 , 1 N HCl and brine respectively, then concentrated to give 2-chloro-*N*-(4-hydroxybutyl)-3,5-dinitrobenzamide (**14**) 1.11 g (35%): mp (EtOAc) 121-124 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.98 (d, $J = 2.7$ Hz, 1H), 8.79 (m, 1 H), 8.52 (d, $J = 2.7$ Hz, 1H), 4.43 (m, 1 H), 3.43 (m, 2H), 3.26 (m, 2 H), 1.54 (m, 4 H); ^{13}C NMR δ 162.6, 148.4, 145.9, 140.4, 128.2, 125.8, 120.4, 60.2, 39.1, 29.8, 25.3. Anal. ($\text{C}_{11}\text{H}_{12}\text{ClN}_3\text{O}_6$) C, H, N.

- A solution of **14** (0.75 g, 2.3 mmol) and Et_3N (2 mL) in *p*-dioxane (30 mL) was treated with N,N -bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc gave the crude product which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-4** (0.99 g, 100%) as yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.71 (d, $J = 2.8$ Hz, 1 H), 8.69 (m, 1 H), 8.27 (d, $J = 2.8$ Hz, 1 H), 4.37 (m, 1 H), 3.70 (m, 4 H), 3.38 (m, 6 H), 3.25 (m, 2 H), 1.56 (m, 2 H), 1.47 (m, 2 H); ^{13}C NMR δ 165.0, 145.7, 145.5, 141.0, 136.4, 127.2, 122.0, 60.2, 54.2, 41.5, 39.2, 29.8, 25.2. HRMS (FAB) Calcd. For $\text{C}_{15}\text{H}_{21}^{35}\text{Cl}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 423.0838. Found; 423.0847.

- A solution of **IIb-4** (0.96 g, 3.04 mmol) and LiBr (5 g) in 3-methyl-2-butanone (15 mL) was heated under reflux for 6 h, then cooled and poured into water. Extraction with EtOAc gave a crude product (<95 % pure), that was re-treated with LiBr (5 g) in 3-methyl-2-butanone for a further 4 h, then worked up and chromatographed on silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 3:1) give **IIb-9** (1.01 g, 87%) as a yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ

8.74 (d, $J = 2.8$ Hz, 1 H), 8.72 (m, 1 H), 8.28 (d, $J = 2.8$ Hz, 1 H), 3.60-3.26 (m, 12 H), 1.58 (m, 2 H), 1.49 (m, 2 H); ^{13}C NMR δ 165.0, 145.6, 145.2, 141.2, 136.5, 127.2, 122.0, 60.2, 54.1, 39.2, 29.9, 29.8, 25.2. HRMS (FAB) Calcd. For $\text{C}_{15}\text{H}_{21}^{79}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 510.9828. Found; 510.9832.

5

Example 13 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-*N*-(5-hydroxypentyl)-3,5-dinitrobenzamide (**IIb-5**) and 2-[bis(2-bromoethyl)amino]-*N*-(5-hydroxypentyl)-3,5-dinitrobenzamide (**IIb-10**). Similar reaction of the acid chloride of **11** with 5-aminopentanol as above gave 2-chloro-*N*-(5-hydroxypentyl)-3,5-dinitrobenzamide (**15**), 1.3 g (39%), mp (EtOAc) 105-108 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.98 (d, $J = 2.7$ Hz, 1H), 8.79 (m, 1 H), 8.50 (d, $J = 2.7$ Hz, 1 H), 4.35 (m, 1 H), 3.39 (m, 2 H), 3.26 (m, 2 H), 1.54 (m, 2 H), 1.44 (m, 2 H), 1.36 (m, 2 H); ^{13}C NMR δ 162.7, 148.4, 145.9, 140.4, 128.2, 125.8, 120.4, 60.5, 39.1, 32.0, 28.4, 22.8. Anal. ($\text{C}_{12}\text{H}_{14}\text{ClN}_3\text{O}_6$) C, H, N.

15 A solution of **15** (0.63 g, 2.3 mmol) and Et_3N (2 mL) in *p*-dioxane (30 mL) was treated with *N,N*-bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc to give the crude product which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-5** (0.82 g, 100%) as yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.73 (d, $J = 2.8$ Hz, 1 H), 8.69 (m, 1 H), 8.28 (d, $J = 2.8$ Hz, 1 H), 4.32 (m, 1 H), 3.70 (m, 4 H), 3.40 (m, 6 H), 3.25 (m, 2 H), 1.55 (m, 2 H), 1.47 (m, 2 H), 1.37 (m, 2 H); ^{13}C NMR δ 165.0, 145.7, 145.5, 141.0, 136.4, 127.2, 122.0, 60.5, 54.2, 41.5, 39.3, 32.0, 28.3, 22.9. HRMS (FAB) Calcd. For $\text{C}_{16}\text{H}_{23}^{35}\text{Cl}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 437.0995. Found; 437.0991.

25 Similar reaction of **IIb-5** (1.3 g) with LiBr gave **IIb-10** (1.35 g, 86%) as a yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, $J = 2.8$ Hz, 1 H), 8.71 (m, 1 H), 8.28 (d, $J = 2.8$ Hz, 1 H), 3.60-3.26 (m, 12 H), 1.55 (m, 2 H), 1.48 (m, 2 H), 1.37 (m, 2 H); ^{13}C NMR δ 165.0, 145.6, 145.2, 141.2, 136.5, 127.2, 122.0, 60.5, 54.1, 39.3, 32.0, 29.8, 28.4, 22.9. HRMS (FAB) Calcd. For $\text{C}_{16}\text{H}_{23}^{79}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 524.9984. Found; 524.9975.

30

Example 14 (Scheme 2d). 2-[Bis(2-chloroethyl)amino]-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (**IIb-6**) and 2-[bis(2-bromoethyl)amino]-*N*-(6-hydroxyhexyl)-3,5-

dinitrobenzamide (IIb-11). Similar reaction of the acid chloride of **11** with 6-aminohexanol as above gave 2-chloro-*N*-(6-hydroxyhexyl)-3,5-dinitrobenzamide (**16**), 0.9 g (26%), mp (EtOAc) 88-91 °C; ¹H NMR [(CD₃)₂SO] δ 8.98 (d, *J* = 2.7 Hz, 1H), 8.78 (m, 1 H), 8.49 (d, *J* = 2.7 Hz, 1 H), 4.32 (m, 1 H), 3.39 (m, 2H), 3.26 (m, 2 H), 1.54 (m, 2 H), 1.44 (m, 2 H), 1.34 (m, 4 H); ¹³C NMR δ 162.7, 148.4, 145.9, 140.4, 128.2, 125.8, 120.4, 60.5, 39.1, 32.3, 28.6, 26.2, 25.1. Anal. (C₁₃H₁₆ClN₃O₆) C, H, N.

A solution of **16** (0.67 g, 2.5 mmol) and Et₃N (2 mL) in *p*-dioxane (30 mL) was treated with *N,N*-bis(2-chloroethyl)amine hydrochloride (1.5 g, 8.0 mmol) at 50 °C for 24 h. The mixture was poured into water and extracted with EtOAc to give the crude product which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (4:1) gave **IIb-6** (0.87 g, 100%) as yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.73 (d, *J* = 2.8 Hz, 1 H), 8.70 (m, 1 H), 8.28 (d, *J* = 2.8 Hz, 1 H), 4.31 (m, 1 H), 3.70 (m, 4 H), 3.38 (m, 6 H), 3.25 (m, 2 H), 1.54 (m, 2 H), 1.40 (m, 2 H), 1.32 (m, 4 H); ¹³C NMR δ 165.0, 145.7, 145.6, 141.0, 136.4, 127.2, 122.0, 60.5, 54.2, 41.5, 39.2, 32.3, 28.5, 26.3, 25.1. HRMS (FAB) Calcd. For C₁₇H₂₅³⁵Cl₂N₄O₆ [M+H⁺] *m/z* 451.1151. Found; 451.1154.

Similar reaction of **IIb-6** (0.97 g) with LiBr gave **IIb-11** (0.96 g, 81%) as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.8 Hz, 1 H), 8.70 (m, 1 H), 8.28 (d, *J* = 2.8 Hz, 1 H), 3.60-3.26 (m, 12 H), 1.54 (m, 2 H), 1.43 (m, 2 H), 1.32 (m, 4 H); ¹³C NMR δ 165.0, 145.6, 145.2, 141.2, 136.5, 127.2, 122.0, 60.6, 54.1, 39.2, 32.4, 29.9, 28.5, 26.3, 25.1. HRMS (FAB) Calcd. For C₁₇H₂₅⁷⁹Br₂N₄O₆ [M+H⁺] *m/z* 539.0141. Found; 539.0135.

Example 15 (Scheme 2e). 2-[Bis(2-bromopropyl)amino]-*N*-(2-hydroxyethyl)-3,5-dinitrobenzamide (IIb-7a). Reaction of 2-chloro-3,5-dinitro-*N*-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]benzamide (**17**) (1.02 g) [For method of preparation see co-pending NZ Application No. 521851] with diisopropanolamine (0.8 g) as above gave 2-[bis(2-hydroxypropyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]benzamide (**18**) (1.29 g, 100%): as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 9.22 (br, 1H), 8.66 (d, *J* = 2.8 Hz, 1H), 8.29 (d, *J* = 2.8 Hz, 1H), 4.99 (m, 1 H), 4.85 (br, 1 H), 4.62 (br, 1 H), 3.94 (m, 2 H), 3.77 (m, 2 H), 3.53 (m, 4H), 3.26 (m, 2 H), 1.48 (m, 10 H), 0.98 (m, 6 H); ¹³C NMR δ 166.5,

147.8, 142.4, 138.2, 132.6, 128.8, 123.8, 98.1, 64.8, 63.5, 61.5, 60.1, 30.1, 25.0, 20.5, 20.2, 19.1. HRMS (FAB) Calcd. For $C_{20}H_{31}N_4O_9$ $[M+H]^+$ m/z 471.2091. Found; 471.2089.

Reaction of **18** with MsCl as above gave 2-[[2-[(methylsulfonyl)oxy]propyl]-4,6-dinitro-6-
5 ([2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]amino)carbonyl]anilino]ethyl methanesulfonate (**19**)
(2.52 g, 100%): as a yellow foam; which was used directly for the next step.

A solution of **19** (2.52 g, 4.03 mmol) in THF (150 mL) was treated with 1 N HCl (100 mL),
and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized
10 with satd. $NaHCO_3$, and extracted with EtOAc (3x80 mL). The combined organic phases were
washed with brine and dried, the solvent was evaporated, and the residue was purified by
chromatography on silica gel, eluting with EtOAc/MeOH(100:1), to give 3-(2-[[2-
hydroxyethyl]amino]carbonyl){3-[(methylsulfonyl)oxy]butyl}-4,6-dinitroanilino)-1-
methylpropyl methanesulfonate (**20**) (0.80 g, 37%): as a yellow foam; 1H NMR $[(CD_3)_2SO]$ δ
15 8.94 (m, 1 H), 8.72 (m, 1 H), 8.35 (m, 1 H), 4.92 (m, 2 H), 3.56 (m, 2 H), 3.30 (m, 6 H), 3.16
(s, 6 H), 1.32 (m, 6 H); ^{13}C NMR δ 165.9, 145.8, 143.4, 139.4, 133.6, 128.0, 123.1, 76.3, 59.2,
57.3, 42.2, 37.7, 18.6. HRMS (FAB) Calcd. For $C_{17}H_{27}N_4O_{12}S_2$ $[M+H]^+$ m/z 543.1067.
Found; 543.1074.

20 Treatment of **20** (0.52 g, 0.96 mmol) with LiBr (0.5 g, 5.2 mmol) in EtOAc (50 mL) at 60 °C
for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether
(from 2:1 to 1:0) gave **IIb-7a** (0.31 g, 62%): as yellow solid: mp (EtOAc/petroleum ether)
127-130 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.91 (m, 1H, CONH), 8.70 (d, J = 2.8 Hz, 1H, H-4), 8.32
(d, J = 2.8 Hz, 1H, H-6), 4.80 (m, 1 H), 4.42 (m, 2 H), 3.55 (m, 4 H), 1.62 (m, 6 H); ^{13}C NMR
25 δ 165.8, 144.8, 143.5, 139.6, 133.6, 128.0, 122.9, 60.6, 59.2, 47.9, 42.2, 23.4. Anal.
($C_{15}H_{20}Br_2N_4O_6$) C, H, N.

Example 16 (Scheme 2f). 2-((2-Bromoethyl)-2-[[2-(hydroxypropyl)amino]carbonyl]-4,6-
dinitroanilino)ethyl methanesulfonate (**IIb-13**). A solution of **13** (1.22 g, 4.0 mmol) in 50
30 mL of CH_2Cl_2 was cooled in an ice-bath, and 3,4-dihydro-2*H*-pyran (1.0 mL) and p-
toluenesulfonic acid (0.1 g) were added. The reaction mixture was stirred for 2 h, then
concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting

with EtOAc/petroleum ether (from 1:2 to 2:1), gave 2-chloro-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (**21**) (1.45 g, 94%): as a pale yellow oil; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.99 (d, $J = 2.7$ Hz, 1 H, H-4), 8.81 (m, 1 H, CONH), 8.51 (d, $J = 2.7$ Hz, 1 H, H-6), 4.57 (m, 1 H), 3.72 (m, 2 H), 3.46-3.25 (m, 4 H), 1.82-1.44 (m, 8 H). ^{13}C NMR δ 162.7, 148.4, 145.9, 140.3, 128.2, 125.8, 120.5, 98.0, 64.2, 61.3, 36.5, 30.2, 28.9, 24.9, 19.1. HRMS (FAB) Calcd. For $\text{C}_{15}\text{H}_{19}^{35}\text{ClN}_3\text{O}_7$ $[\text{M}+\text{H}^+]$ m/z 388.0912. Found; 388.0915.

Reaction of **21** (1.45 g, 3.75 mmol) with diethanolamine (1.67 g) as above gave 2-[bis(2-hydroxyethyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (**22**) (1.62 g, 95%): as a yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.96 (m, 1H, CONH), 8.66 (d, $J = 2.8$ Hz, 1H, H-4), 8.31 (d, $J = 2.8$ Hz, 1H, H-6), 4.95 (m, 2H), 4.56 (m, 1H), 3.79-3.16 (m, 14H), 1.80-1.45 (m, 8 H); ^{13}C NMR δ 166.2, 148.1, 143.6, 139.3, 133.8, 128.9, 123.8, 98.5, 64.8, 61.7, 58.5, 54.6, 37.3, 30.6, 29.2, 25.4, 19.6. HRMS (FAB) Calcd. For $\text{C}_{19}\text{H}_{29}\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 457.1935. Found; 457.1939.

Reaction of **22** (1.62 g, 3.55 mmol) with MsCl (2 mL) as above gave 2-[(2-(methanesulfonyl)ethoxy)ethyl]-4,6-dinitro-6-([2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]-amino)carbonylanilino]ethyl methanesulfonate (**23**) (2.17 g, 100%): as a yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.71 (d, $J = 2.8$ Hz, 1H), 8.71 (m, 1H), 8.31 (d, $J = 2.8$ Hz, 1H), 4.26 (m, 4 H), 3.71-3.37 (m, 10 H), 3.13 (s, 6 H), 3.10 (m, 2 H), 1.82-1.43 (m, 8 H); ^{13}C NMR δ 165.1, 146.3, 145.4, 140.9, 135.9, 127.4, 122.2, 98.0, 67.2, 64.3, 51.4, 45.7, 36.5, 30.2, 28.7, 24.9, 19.1, 8.5. HRMS (FAB) Calcd. For $\text{C}_{21}\text{H}_{33}\text{N}_4\text{O}_{13}\text{S}_2$ $[\text{M}+\text{H}^+]$ m/z 613.1486. Found; 613.1481.

A solution of **23** (2.95 g, 3.55 mmol) in THF (120 mL) was treated with 1 N HCl (80 mL), and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized with satd. NaHCO_3 , and extracted with EtOAc (3x80 mL). The combined organic phases were washed with brine and dried, the solvent was evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH(100:1), to give **24** (1.4 g, 75%): as a yellow solid: mp (EtOAc/petroleum ether) 130-133 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, $J = 2.8$ Hz, 1H), 8.72 (m, 1H), 8.32 (d, $J = 2.8$ Hz, 1H), 4.29 (m, 4 H), 3.47 (m, 8 H), 3.14 (s, 6 H), 1.71 (m, 2 H); ^{13}C NMR δ 165.2, 146.3, 145.3, 140.8, 135.9, 127.5, 122.3, 67.3, 58.4, 51.4, 36.8, 36.5, 31.7. Anal. ($\text{C}_{16}\text{H}_{24}\text{N}_4\text{O}_{12}\text{S}_2$) C, H, N.

Treatment of **24** (0.25 g, 0.45 mmol) with LiBr (53 mg, 0.61 mmol) in EtOAc (50 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave **II-13** (0.16 g, 66%): as yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.8 Hz, 1H), 8.73 (m, 1H), 8.31 (d, *J* = 2.8 Hz, 1H), 4.28 (m, 2 H), 3.65-3.44 (m, 10 H), 3.13 (s, 3 H), 1.70 (m, 2 H); ¹³C NMR δ 165.1, 145.7, 145.4, 141.0, 136.2, 127.3, 122.1, 67.5, 58.4, 51.1, 36.7, 36.5, 31.7, 29.6. HRMS (FAB) Calcd. For C₁₅H₂₂⁷⁹BrN₄O₉S [M+H⁺] *m/z* 513.0291. Found; 513.0281.

Example 17 (Scheme 2g). 2-((2-bromoethyl)-2-[[2-hydroxyethyl)amino]carbonyl]-4,6-dinitroanilino)ethyl methanesulfonate (**IIb-12**). Solid **IIb-7** (300 mg, 0.62 mmol) and silver methanesulfonate (130 mg, 0.65 mmol) in dry MeCN (15 mL) were heated under reflux for 3 h, then cooled and filtered. The solid AgBr was washed with EtOAc to give a 98% yield of AgBr. The solvent was removed at reduced pressure and the residue was separated by chromatography on silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 1:0), to give successively:

- starting material (**IIb-7**) (28 mg, 9%).

- **IIb-12** (123 mg, 38%) as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1 H, CONH), 8.74 (d, *J* = 2.7 Hz, 1 H, H-4), 8.36 (d, *J* = 2.7 Hz, 1 H, H-6), 4.28 (m, 2 H, CH₂OMs), 3.58 (m, 4 H), 3.44 (m, 4 H), 3.14 (s, 3 H, OSO₂CH₃); ¹³C NMR δ 165.3, 145.8, 145.2, 140.9, 135.1, 127.5, 122.2, 67.5, 59.2, 54.2, 51.0, 42.1, 36.4, 29.7; HRMS *m/z* required for C₁₄H₂₀⁷⁹BrN₄O₉S 499.01344; Found 499.01324.

The column was finally eluted with EtOAc/MeOH (9:1) to give 2-(2-[[2-hydroxyethyl)amino]carbonyl} {2-[(methylsulfonyl)oxy]ethyl}-3,5-dinitroanilino)ethyl methanesulfonate **25** (159 mg, 53%) as a yellow solid: mp 128-132 °C (EtOAc/petroleum ether); ¹H NMR [(CD₃)₂SO] δ 8.78 (m, 1 H, CONH), 8.74 (d, *J* = 2.7 Hz, 1 H, H-4), 8.36 (d, *J* = 2.7 Hz, 1 H, H-6), 4.29 (m, 4 H, 2xCH₂OMs), 3.56 (m, 2 H), 3.45 (m, 6 H), 3.14 (s, 6 H, 2xOSO₂CH₃); ¹³C NMR δ 165.4, 146.3, 145.1, 140.6, 135.8, 127.6, 122.3, 67.3, 59.2, 51.3, 42.1, 36.4; HRMS: C₁₅H₂₃N₄O₁₂S₂ requires *m/z* 515.0754. Found: 515.0744.

Example 18 (Scheme 2h). 2-((2-Chloroethyl)-2-[[2-hydroxyethyl)amino]carbonyl]-4,6-dinitroanilino)ethyl methanesulfonate (**IIb-2m**). A solution of **25** (5.3 g, 10.3 mmol) in

DMF (100 mL) was treated with LiCl (524 mg, 12.4 mmol) at 60 °C for 2 h, and then cooled, poured into dilute HCl and extracted with EtOAc (3x150 mL). Workup and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether from 1:1 to 1:0, gave **IIb-2** (2.4 g, 59%), and then **IIb-2m** (1.94 g, 41%) as yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1H, CONH), 8.74 (d, J= 2.7 Hz, 1H, H-4), 8.36 (d, J=2.7 Hz, 1H, H-6), 4.28 (m, 2H, -CH₂O-Ms), 3.58 (m, 4H), 3.44 (m, 4H), 3.14 (s, 3 H, -OSO₂CH₃); ¹³C NMR δ 165.3, 145.8, 145.2, 140.9, 135.1, 127.5, 122.2, 67.5, 59.2, 54.2, 51.0, 42.1, 36.4, 29.7.

Example 19 (Scheme 2i). 2-[Bis(2-iodoethyl)amino]-N-(2-hydroxyethyl)-3,5-

dinitrobenzamide (IIb-14) and 2-((2-iodoethyl)-2-[(2-hydroxyethyl)amino]carbonyl)-4,6-dinitroanilino)ethyl methanesulfonate (IIb-15). Treatment of **25** [For method of preparation see co-pending NZ Application No. 521851] (6.7 g, 13.0 mmol) with NaI (2.9 g, 20 mmol) in EtOAc (200 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave **IIb-14** (3.3 g, 44%) as a yellow solid: mp (EtOAc/petroleum ether) 129-131 °C; ¹H NMR [(CD₃)₂SO] δ 8.72 (d, J= 2.8 Hz, 1 H, H-4), 8.70 (m, 1 H, CONH), 8.32 (d, J= 2.8 Hz, 1 H, H-6), 4.80 (m, 1 H), 3.55 (m, 2 H), 3.43 (m, 4 H), 3.31 (m, 6 H); ¹³C NMR δ 165.3, 145.2, 144.7, 141.0, 136.3, 127.3, 122.0, 59.3, 54.7, 42.1, 2.94. Anal (C₁₃H₁₆N₄I₂O₆) C, H, N.

Later eluates gave **IIb-15** (1.35 g, 19%) as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, J= 2.8 Hz, 1H, H-4), 8.74 (m, 1H, CONH), 8.34 (d, J= 2.8 Hz, 1 H, H-6), 4.28 (m, 2 H), 3.56 (m, 2 H), 3.43 (m, 2 H), 3.31 (m, 6 H), 3.13 (s, 3 H); ¹³C NMR δ 165.3, 145.5, 145.2, 140.8, 136.1, 127.4, 122.1, 67.5, 59.2, 55.4, 50.6, 42.1, 36.5, 2.6. HRMS (FAB) Calcd. For C₁₄H₂₀IN₄O₉S [M+H⁺] m/z 546.9996. Found; 546.9997.

Example 20 (Scheme 2j). 3-[Bis(2-bromoethyl)amino]-N-(2-hydroxyethyl)-2,6-

dinitrobenzamide (IIc-7) and 2-((2-bromoethyl)-3-[(2-hydroxyethyl)amino]carbonyl)-2,6-dinitroanilino)ethyl methanesulfonate (IIc-12). Treatment of 3-(3-[(2-hydroxyethyl)amino]carbonyl){3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate (**26**) [for method of preparation see NZ Application No. 521851] (310 mg, 0.6 mmol) in EtOAc (50 mL) with LiBr (78 mg, 0.9 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave

IIc-7 (70 mg, 25%) as a foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.80 (m, 1H, CONH), 8.24 (d, $J = 9.4$ Hz, 1H), 7.63 (d, $J = 9.4$ Hz, 1H), 4.66 (m, 1 H), 3.70 (m, 4 H), 3.60 (m, 4 H), 3.45 (m, 2 H), 3.22 (m, 2 H); ^{13}C NMR δ 161.4, 145.8, 140.2, 137.5, 129.2, 127.6, 122.6, 59.0, 52.6, 41.7, 30.0. HRMS (FAB) Calcd. For $\text{C}_{13}\text{H}_{17}^{79}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 482.9515. Found; 482.9508.

5

Further elution with EtOAc/MeOH (50:2) gave **IIc-12** (118 mg, 39%): mp. 94-97 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.80 (m, 1H, CONH), 8.25 (d, $J = 9.4$ Hz, 1H), 7.64 (d, $J = 9.4$ Hz, 1H), 4.67 (m, 1 H), 4.27 (m, 2 H), 3.63 (m, 4 H), 3.57 (m, 2 H), 3.45 (m, 2 H), 3.26 (m, 2 H), 3.15 (s, 3 H); ^{13}C NMR δ 161.4, 146.2, 140.5, 137.7, 129.2, 127.5, 122.9, 66.8, 59.0, 50.0, 41.7, 36.6, 29.9. Anal. ($\text{C}_{14}\text{H}_{19}\text{BrN}_4\text{O}_9\text{S}$) C, H, N.

10

Example 21 (Scheme 2j). 3-[Bis(2-bromoethyl)amino]-*N*-(3-hydroxypropyl)-2,6-dinitrobenzamide (**IIc-8**) and 2-((2-bromoethyl)-3-((3-hydroxypropyl)amino)carbonyl)-2,6-dinitroanilino)ethyl methanesulfonate (**IIc-13**). Treatment of 3-(3-((3-hydroxypropyl)amino)carbonyl) {3-[(methanesulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate (**27**) [for method of preparation see co-pending NZ Application No. 521851] (716 mg, 1.36 mmol) in EtOAc (200 mL) with LiBr (175 mg, 2.0 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave **IIc-8** (289 mg, 42%) as a yellow solid; mp (EtOAc/petroleum ether) 142-144 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.75 (t, $J = 5.8$ Hz, 1 H, CONH), 8.23 (d, $J = 9.4$ Hz, 1 H, H-5), 7.62 (d, $J = 9.4$ Hz, 1 H, H-6), 4.47 (m, 1 H, CHOH), 3.68 (m, 4 H), 3.57 (m, 4 H), 3.43 (m, 2 H), 3.20 (m, 2 H), 1.60 (m, 2 H); ^{13}C NMR δ 161.2, 146.9, 140.2, 137.5, 129.4, 127.7, 122.6, 58.3, 52.6, 36.4, 31.6, 30.1. HRMS (FAB) Calcd. For $\text{C}_{14}\text{H}_{19}^{79}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 496.9671. Found: 496.9667.

20

25

Further elution with EtOAc/MeOH (50:2) gave **IIc-13** (270 mg, 39%): mp. 115-117 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.75 (t, $J = 5.8$ Hz, 1 H, CONH), 8.24 (d, $J = 9.4$ Hz, 1 H, H-5), 7.64 (d, $J = 9.4$ Hz, 1 H, H-6), 4.43 (m, 1 H, CHOH), 4.27 (m, 2 H, CH_2OMs), 3.66 (m, 4 H, $2\times\text{CH}_2\text{N}$), 3.59 (m, 2 H), 3.44 (m, 2 H), 3.22 (m, 2 H), 3.15 (s, 3 H, CH_3SO_3), 1.60 (m, 2 H); ^{13}C NMR δ 161.1, 146.2, 140.5, 137.7, 129.2, 127.6, 122.9, 66.8, 58.2, 52.9, 50.0, 36.6, 36.4, 31.6, 30.0. Anal. ($\text{C}_{15}\text{H}_{21}\text{BrN}_4\text{O}_9\text{S}$) C, H, N.

30

Example 22 (Scheme 2j). 3-[Bis(2-bromoethyl)amino]-*N*-(4-hydroxybutyl)-2,6-dinitrobenzamide (**IIc-9**) and 2-((2-bromoethyl)-3-[[4-hydroxybutyl)amino]carbonyl]-2,6-dinitroanilino)ethyl methanesulfonate (**IIc-14**). Treatment of 3-(3-[[4-hydroxybutyl)amino]carbonyl]{3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate (**28**) [for method of preparation see NZ Application No. 521851] (500 mg, 0.92 mmol) in EtOAc (100 mL) with LiBr (110 mg, 1.4 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave **IIc-9** (100 mg, 21%) as a foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.73 (m, 1H, CONH), 8.25 (d, $J = 9.4$ Hz, 1H), 7.63 (d, $J = 9.4$ Hz, 1H), 4.38 (m, 1 H), 3.69 (m, 4 H), 3.57 (m, 4 H), 3.40 (m, 2 H), 3.14 (m, 2 H), 1.47 (m, 4 H); ^{13}C NMR δ 161.0, 145.8, 140.2, 137.6, 129.3, 127.6, 122.6, 60.2, 52.6, 30.0, 29.6, 24.8. HRMS (FAB) Calcd. For $\text{C}_{15}\text{H}_{20}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$ m/z 510.9828. Found; 510.9819.

Further elution with EtOAc/MeOH (50:2) gave **IIc-14** (117 mg, 30%): mp. 114-117 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (m, 1 H, CONH), 8.25 (d, $J = 9.4$ Hz, 1 H), 7.65 (d, $J = 9.4$ Hz, 1 H), 4.37 (m, 1 H), 4.27 (m, 2 H), 3.65 (m, 4 H), 3.57 (m, 2 H), 3.35 (m, 2 H), 3.16 (m, 2 H), 3.15 (s, 3 H), 1.47 (m, 4 H); ^{13}C NMR δ 160.0, 146.1, 140.6, 137.8, 129.2, 127.5, 122.9, 66.8, 60.2, 52.9, 50.0, 36.6, 29.9, 29.6, 24.9. Anal. ($\text{C}_{16}\text{H}_{23}\text{BrN}_4\text{O}_9\text{S}$) C, H, N.

Example 23 (Scheme 2k). 3-(3-[[3-hydroxypropyl)amino]carbonyl]{3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate (**27**) and 2-((2-bromoethyl)-3-[[3-hydroxypropyl)amino]carbonyl]-2,6-dinitroanilino)ethyl methanesulfonate (**IIc-13**). Solid **IIc-8** (2.15 g, 4.3 mmol) was added to a hot solution of silver methanesulfonate (0.992 g, 4.9 mmol) in dry MeCN (40 mL). The mixture was heated under reflux for 3 h, then cooled and filtered. The solvent was removed at reduced pressure and the residue was separated by chromatography on silica gel, eluting with EtOAc/petroleum ether (from 1:1 to 1:0), to give successively **IIc-13** (0.5 g, 25%), **IIc-8** (0.3 g, 14%) and **27** (0.4 g, 18%).

Example 24 (Scheme 2k). 2-((2-chloroethyl)-3-[[3-hydroxypropyl)amino]carbonyl]-2,6-dinitroanilino)ethyl methanesulfonate (**IIc-6**). A solution of **27** (9.0 g, 17.0 mmol) in DMF (110 mL) was treated with LiCl (860 mg, 20.4 mmol) at 60 °C for 2 h, then cooled, poured

into dilute HCl, and extracted with EtOAc (3x150 mL). Workup and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether from 1:1 to 1:0, gave **IIc-6** (4.0 g, 50%) as yellow crystals: mp 104-109°C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.75 (t, $J = 5.8$ Hz, 1H, CONH), 8.24 (d, $J = 9.4$ Hz, 1H, H-5), 7.64 (d, $J = 9.4$ Hz, 1H, H-6), 4.44 (m, 1H, CHOH), 4.26 (m, 2H), 3.72 (m, 2H), 3.65 (m, 2H), 3.59 (m, 2H), 3.43 (m, 2H), 3.20 (m, 2H), 3.15 (s, 3H), 1.60 (m, 2H); ^{13}C NMR δ 161.1, 146.4, 140.5, 137.7, 129.2, 127.6, 122.9, 66.8, 58.2, 52.9, 50.1, 41.4, 36.6, 36.4, 31.6. Anal. ($\text{C}_{15}\text{H}_{21}\text{ClN}_4\text{O}_9\text{S}$) C, H, N.

Example 25 (Scheme 2k): 2-((2-Iodoethyl)-3-[(3-hydroxypropyl)amino]carbonyl)-2,6-dinitroanilino)ethyl methanesulfonate (IIc-15). A solution of **27** (5.28 g, 10.0 mmol) in EtOAc (250 mL) was treated with NaI (1.8 g, 12.0 mmol) at 60 °C for 2 h, and the product was chromatographed on silica gel, eluting with EtOAc/petroleum ether from 1:2 to 1:0, to give **IIc-15** (2.29 g, 41%) as yellow crystals: mp 100-103°C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 10.05 (s, 1H), 7.40 (d, $J = 11.5$ Hz, 1H), 7.09 (s, 1H), 6.70 (d, $J = 11.5$ Hz, 1H), 2.50 (m, 2H), 2.21 (m, 2H), 2.03 (s, 3H), 1.52 (m, 4H); ^{13}C NMR δ 161.1, 145.8, 140.5, 137.7, 129.2, 127.6, 122.9, 66.8, 58.2, 53.9, 49.9, 41.4, 36.6, 36.4, 31.6. Anal. ($\text{C}_{15}\text{H}_{21}\text{IN}_4\text{O}_9\text{S}$) C, H, N.

Preparation of Phosphates (Scheme 3)

Example 26. 2-[[2-[Bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate (Ib-7P). A solution of alcohol **Ib-7** (2.58 g, 5.33 mmol) and di-*tert*-butyl diethylphosphoramidite (93%, 2.07 mL, 6.93 mmol) in dry DMF (20 mL) under N_2 was treated with 1*H*-tetrazole (3 wt. % in CH_3CH , 55.1 mL, 18.7 mmol) and stirred at 20 °C for 1.5 h. The reaction mixture was then cooled to -50 °C and a solution of 3-chloroperoxybenzoic acid (55%, 2.68 g, 8.54 mmol) was rapidly added such that the temperature was kept below -5 °C. The reaction mixture was warmed to room temperature and diluted with CH_2Cl_2 (150 mL). The solution was washed with 5% aqueous $\text{Na}_2\text{S}_2\text{O}_5$ (2 x 50 mL), 10% aqueous NaHCO_3 (2 x 50 mL), water (2 x 50 mL), dried, concentrated under reduced pressure below 30 °C and the residue was shaken with *i*- Pr_2O /hexane and refrigerated. The resulting solid was purified by chromatography on silica gel, eluting with CH_2Cl_2 /EtOAc, followed by recrystallisation from CH_2Cl_2 /hexane (below 40 °C) to give di-*tert*-butyl 2-[[2-[bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl phosphate (**Ib-7E**) (2.59 g, 72%) as

an unstable yellow solid: mp 99-101 °C (dec); ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.93 (t, J = 5.6 Hz, 1 H), 8.76 (d, J = 2.8 Hz, 1 H), 8.33 (d, J = 2.8 Hz, 1 H), 4.01 (g, J = 6.1 Hz, 2 H), 3.62-3.42 (m, 10 H), 1.43 (s, 18 H). HRMS (FAB) calcd for $\text{C}_{21}\text{H}_{34}^{79}\text{Br}_2\text{N}_4\text{O}_9\text{P}$ (MH^+) m/z 675.0430 found 675.0398; calcd for $\text{C}_{21}\text{H}_{34}^{79}\text{Br}^{81}\text{BrN}_4\text{O}_9\text{P}$ (MH^+) m/z 677.0410, found 677.0397; calcd for $\text{C}_{21}\text{H}_{34}^{81}\text{Br}_2\text{N}_4\text{O}_9\text{P}$ (MH^+) m/z 679.0389, found 679.0398. Anal. ($\text{C}_{21}\text{H}_{33}\text{Br}_2\text{N}_4\text{O}_9\text{P}$).

A solution of **Ib-7E** (2.80 g, 4.14 mmol) and TFA (15 mL) in dry CH_2Cl_2 (15 mL) was stirred at 20 °C for 1 h, then concentrated under reduced pressure. Residual TFA was removed azeotropically with CH_3CN (2 x) and the resulting residue was dissolved in EtOAc. Addition of excess hexane precipitated a semisolid which was dried under high vacuum at 20 °C to give **Ib-7P** (98%) as a yellow foam. ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.93 (t, J = 5.6 Hz, 1 H), 8.75 (d, J = 2.8 Hz, 1 H), 8.36 (d, J = 2.8 Hz, 1 H), 3.97 (q, J = 6.3 Hz, 2 H), 3.62-3.43 (m, 10 H). HRMS (FAB) calcd for $\text{C}_{13}\text{H}_{18}^{79}\text{Br}_2\text{N}_4\text{O}_9\text{P}$ (MH^+) m/z 562.9178, found 562.9171; calcd for $\text{C}_{13}\text{H}_{18}^{79}\text{Br}^{81}\text{BrN}_4\text{O}_9\text{P}$ (MH^+) m/z 564.9158, found 564.9152; calcd for $\text{C}_{13}\text{H}_{18}^{81}\text{Br}_2\text{N}_4\text{O}_9\text{P}$ (MH^+) m/z 566.9137, found 566.9121. Treatment of diacid **Ib-7P** with NaHCO_3 (2.0 equiv.) gave the disodium salt.

Example 27. 3-[[5-[Bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl dihydrogen phosphate (Ia-3P). Similar phosphorylation of **Ia-3**, followed by chromatography of the product on silica gel and elution with $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (2:3), gave di-*tert*-butyl 3-[[5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzoyl]amino]propyl phosphate (**Ia-3E**) (76%) as a yellow solid: mp (EtOAc/*i*-Pr₂O/hexane) 120-121 °C (dec); ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.70 (t, J = 5.6 Hz, 1 H), 8.55 (s, 1 H), 7.45 (s, 1 H), 3.96 (q, J = 6.7 Hz, 2 H), 3.82 (t, J = 5.8 Hz, 4 H), 3.69 (t, J = 5.8 Hz, 4 H), 3.34 (after D_2O exchange, t, J = 6.8 Hz, 2 H), 1.86 (pent, J = 6.6 Hz, 2 H), 1.42 (s, 18 H). Anal. ($\text{C}_{22}\text{H}_{35}\text{Cl}_2\text{N}_4\text{O}_9\text{P}$) C, H, N.

Similar treatment of ester **Ia-3E** with TFA gave diacid **Ia-3P** (99%) as a hygroscopic yellow solid. ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.71 (t, J = 5.6 Hz, 1 H), 8.54 (s, 1 H), 7.45 (s, 1 H), 3.92 (q, J = 6.7 Hz, 2 H), 3.82 (t, J = 5.8 Hz, 4 H), 3.69 (t, J = 5.8 Hz, 4 H), 3.31 (q, J = 6.5 Hz, 2 H), 1.84 (pent, J = 6.6 Hz, 2 H). HRMS (FAB) Calcd. for $\text{C}_{14}\text{H}_{20}^{35}\text{Cl}_2\text{N}_4\text{O}_9\text{P}$ $[\text{M}+\text{H}]^+$ m/z 489.0345; found 489.0344. Calcd. for $\text{C}_{14}\text{H}_{20}^{35}\text{Cl}^{37}\text{ClN}_4\text{O}_9\text{P}$ $[\text{M}+\text{H}]^+$ m/z 491.0316; found 491.0317.

Calcd. for $C_{14}H_{20}^{37}Cl_2N_4O_9P$ $[M+H]^+$ m/z 493.0286; found 493.0312. Treatment of diacid **I-3P** with $NaHCO_3$ (2:0 equiv) gave the disodium salt.

Example 28. 3-[[5-[Bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl

dihydrogen phosphate (Ia-8P). Similar phosphorylation of **IIa-8**, followed by chromatography of the product on silica gel and elution with $CH_2Cl_2/EtOAc$ (1:1), gave di-*tert*-butyl 3-[[5-[bis(2-bromoethyl)amino]-2,4-dinitrobenzoyl]amino]propyl phosphate (**Ia-8E**) (66%) as a yellow solid: mp ($EtOAc/i-Pr_2O$ /hexane) 110-111°C (dec). 1H NMR ($(CD_3)_2SO$) δ 8.70 (t, $J = 5.6$ Hz, 1 H), 8.55 (s, 1 H), 7.44 (s, 1 H), 3.96 (q, $J = 6.7$ Hz, 2 H), 3.79-3.63 (m, 84 H), 3.35 (after D_2O exchange, t, $J = 6.8$ Hz, 2 H), 1.86 (pent, $J = 6.6$ Hz, 2 H), 1.42 (s, 18 H). Anal. ($C_{22}H_{35}Br_2N_4O_9P$) C, H, N.

Similar treatment of ester **Ia-8E** with TFA gave diacid **Ia-8P** (99%) as a hygroscopic yellow solid. 1H NMR [$(CD_3)_2SO$] δ 8.71 (t, $J = 5.6$ Hz, 1 H), 8.55 (s, 1 H), 7.43 (s, 1 H), 3.93 (q, $J = 6.7$ Hz, 2 H), 3.79-3.63 (m, 8 H), 3.31 (q, $J = 6.5$ Hz, 2 H), 1.85 (pent, $J = 6.6$ Hz, 2 H). HRMS (FAB) calcd for $C_{14}H_{20}^{79}Br_2N_4O_9P$ (MH^+) m/z 576.9335, found 576.9314; calcd for $C_{14}H_{20}^{79}Br^{81}BrN_4O_9P$ (MH^+) m/z 578.9314, found 578.9305; calcd for $C_{14}H_{20}^{81}Br_2N_4O_9P$ (MH^+) m/z 580.9294, found 580.9297. Treatment of diacid **Ia-8P** with $NaHCO_3$ (2.0 equiv.) gave the disodium salt.

Example 29. 2-[[2-[Bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate (Ib-2P). Similar phosphorylation of **IIb-2**, followed by chromatography of the

product on silica gel and elution with $CH_2Cl_2/EtOAc$ (13:7), gave di-*tert*-butyl 2-[[2-[bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl phosphate (**Ib-2E**) (72%) as a yellow solid: mp ($EtOAc/i-Pr_2O$ /hexane) 107-108 °C (dec); 1H NMR [$(CD_3)_2SO$] δ 8.90 (t, $J = 5.6$ Hz, 1 H), 8.75 (d, $J = 2.8$ Hz, 1 H), 8.33 (d, $J = 2.8$ Hz, 1 H), 4.01 (q, $J = 6.1$ Hz, 2 H), 3.72 (t, $J = 6.8$ Hz, 4 H), 3.53 (q, $J = 5.5$ Hz, 2 H), 3.43 (t, $J = 6.8$ Hz, 4 H), 1.43 (s, 18 H). Anal. ($C_{21}H_{33}Cl_2N_4O_9P$) C, H, N, P. CRL 11363.

Similar treatment of ester **Ib-2E** with TFA gave diacid **Ib-2P** (98%) as a yellow foam. 1H NMR [$(CD_3)_2SO$] δ 8.89 (t, $J = 5.6$ Hz, 1 H), 8.74 (d, $J = 2.8$ Hz, 1 H), 8.36 (d, $J = 2.8$ Hz, 1 H), 3.98 (q, $J = 6.2$ Hz, 2 H), 3.72 (t, $J = 6.7$ Hz, 4 H), 3.51 (q, $J = 5.6$ Hz, 2 H), 3.43 (t, $J =$

6.7 Hz, 4 H). HRMS (FAB) Calcd. for $C_{13}H_{18}^{35}Cl_2N_2O_9P$ $[M+H]^+$ m/z 475.0189; found 475.0189. Calcd. for $C_{13}H_{18}^{35}Cl^{37}ClN_2O_9P$ $[M+H]^+$ m/z 477.0159; found 477.0167. Calcd. for $C_{13}H_{18}^{35}Cl_2N_2O_9P$ $[M+H]^+$ m/z 479.0130; found 479.0160. Treatment of diacid **Ib-2P** with $NaHCO_3$ (1.0 equiv.) gave the monosodium salt.

5

Example 30. 2-[(2-Chloroethyl)-2,4-dinitro-6-[[[2-(phosphonoxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate (**Ib-2mP**). Similar phosphorylation of **Ib-2m**, followed by chromatography of the product on silica gel and elution with EtOAc/MeOH (19:1), gave 2-[(2-chloroethyl)-2-(6-*tert*-butoxy-8,8-dimethyl-6-oxido-5,7-dioxo-2-aza-6-phosphanon-1-onyl)-4,6-dinitroanilino]ethyl methanesulfonate (**Ib-2mE**) (80%) as a yellow foam. 1H NMR $[(CD_3)_2SO]$ δ 8.94 (t, $J = 5.6$ Hz, 1 H), 8.75 (d, $J = 2.8$ Hz, 1 H), 8.34 (d, $J = 2.8$ Hz, 1 H), 4.28 (t, $J = 5.4$ Hz, 2 H), 4.02 (q, $J = 6.2$ Hz, 2 H), 3.74-3.43 (m, 8 H), 3.13 (s, 3 H), 1.43 (s, 18 H). ^{13}C NMR δ 265.6, 146.2, 145.3, 140.8, 135.6, 127.5, 122.4, 81.7, 67.5, 64.2, 54.3, 51.3, 41.4, 36.5, 29.5.

15

Similar treatment of ester **Ib-2mE** with TFA gave diacid **Ib-2mP** (68%) as a yellow solid. Mp (EtOAc/ CH_2Cl_2): 132-134 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.92 (t, $J = 5.6$ Hz, 1 H), 8.74 (d, $J = 2.8$ Hz, 1 H), 8.37 (d, $J = 2.8$ Hz, 1 H), 4.29 (t, $J = 5.4$ Hz, 2 H), 3.98 (q, $J = 6.0$ Hz, 2 H), 3.58-3.40 (after D_2O exchange, m, 8 H), 3.13 (s, 2 H). ^{13}C NMR δ 165.5, 146.1, 145.3, 140.8, 135.7, 127.6, 122.3, 67.5, 63.3, 63.2, 54.3, 51.3, 41.3, 36.5. Anal. ($C_{14}H_{20}ClN_4O_{12}PS$) C, H, N.

20

Example 31. 2-({2-[Bis(2-bromopropyl)amino]-3,5-dinitrobenzoyl}amino)ethyl dihydrogen phosphate (**Ib-7aP**). Similar phosphorylation of alcohol **Ib-7a** (0.67 g, 1.3 mmol) with di-*tert*-butyldiethylphosphoramidite (93%, 489 mg, 2.0 mmol), followed by flash column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1) gave **Ib-7aE** as a yellow solid (0.74 g, 81%): mp (EtOAc/petroleum ether) 121-123 °C; 1H NMR $[(CD_3)_2SO]$ δ 9.09 (m, 1 H), 8.73 (m, 1 H), 8.32 (m, 1 H), 4.44 (m, 2 H), 4.00 (m, 2 H), 3.39 (m, 2 H), 3.60 (m, 4 H), 1.62 (m, 6 H), 1.44 (s, 18 H). ^{13}C NMR δ 165.9, 144.8, 143.6, 139.6, 133.2, 128.0, 123.1, 81.6, 64.0, 60.4, 39.9, 29.4, 23.5. Anal. ($C_{23}H_{37}Br_2N_4O_9P$) C, H, N.

25

30

Similar treatment of **Ib-7aE** (100 mg) with TFA (6 mL), followed by crystallization from $\text{CH}_2\text{Cl}_2/\text{EtOAc}$, gave **Ib-7aP** as a yellow solid (70 mg, 85%): mp 157-161 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 9.07 (m, 1 H), 8.72 (m, 1 H), 8.36 (m, 1 H), 4.43 (m, 2 H), 4.00 (m, 2 H), 3.52 (m, 6 H), 1.62 (m, 6 H). ^{13}C NMR δ 165.9, 144.8, 143.6, 139.7, 133.4, 128.1, 123.1, 63.2, 60.4, 47.9, 39.9, 23.5. Anal. ($\text{C}_{15}\text{H}_{21}\text{Br}_2\text{N}_4\text{O}_9\text{P}$) C, H, N.

Example 32. 2-[(2-Bromoethyl)-2,4-dinitro-6-[[[2-(phosphonoxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate (Ib-12P). Similar phosphorylation of **Ia-12**, followed by chromatography of the product on silica gel and elution with EtOAc/MeOH (19:1), gave 2-[(2-bromoethyl)-2-(6-*tert*-butoxy-8,8-dimethyl-6-oxido-5,7-dioxa-2-aza-6-phosphanon-1-onyl)-4,6-dinitroanilino]ethyl methanesulfonate (**Ib-12E**) (66%) as a yellow foam. ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.94 (t, $J = 5.6$ Hz, 1 H), 8.75 (d, $J = 2.8$ Hz, 1 H), 8.34 (d, $J = 2.8$ Hz, 1 H), 4.28 (t, $J = 5.4$ Hz, 2 H), 4.02 (q, $J = 6.2$ Hz, 2 H), 3.62-3.43 (m, 8 H), 3.13 (s, 3 H), 1.43 (s, 18 H). HRMS (FAB) calcd for $\text{C}_{22}\text{H}_{37}^{79}\text{BrN}_4\text{O}_{12}\text{PS}$ $[\text{M}+\text{H}]^+$ m/z 693.1029; found 693.1010.

Similar treatment of ester **Ib-12E** with TFA gave diacid **Ib-12P** (98%) as a yellow foam. ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.92 (t, $J = 5.6$ Hz, 1 H), 8.74 (d, $J = 2.8$ Hz, 1 H), 8.37 (d, $J = 2.8$ Hz, 1 H), 4.28 (t, $J = 5.4$ Hz, 2 H), 3.98 (q, $J = 6.0$ Hz, 2 H), 3.58-3.40 (after D_2O exchange, m, 8 H), 3.13 (s, 2 H). HRMS (FAB) calcd for $\text{C}_{14}\text{H}_{21}^{79}\text{BrN}_4\text{O}_{12}\text{PS}$ $[\text{M}+\text{H}]^+$ m/z 578.9798; found 578.9784; calcd for $\text{C}_{14}\text{H}_{21}^{81}\text{Br}^{81}\text{BrN}_4\text{O}_{12}\text{PS}$ $[\text{M}+\text{H}]^+$ m/z 580.9777; found 580.9784.

Treatment of diacid **Ib-12P** with NaHCO_3 (1.0 equiv) gave the monosodium salt.

Example 33. 2-[[2-[Bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl dihydrogen phosphate (Ib-14P). Similar phosphorylation of **Ib-14**, followed by chromatography of the product on silica gel and elution with $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (3:1), gave di-*tert*-butyl 2-[[2-[bis(2-iodoethyl)amino]-3,5-dinitrobenzoyl]amino]ethyl phosphate (**Ib-14E**) (67%) as a yellow solid: mp ($\text{CH}_2\text{Cl}_2/i\text{-Pr}_2\text{O}/\text{hexane}$) 108-110 °C (dec); ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.91 (t, $J = 5.6$ Hz, 1 H), 8.74 (d, $J = 2.8$ Hz, 1 H), 8.30 (d, $J = 2.8$ Hz, 1 H), 4.01 (q, $J = 6.3$ Hz, 2 H), 3.53 (q, $J = 5.7$ Hz, 2 H), 3.45 (t, $J = 7.8$ Hz, 4 H), 3.24 (after D_2O exchange, t, $J = 7.6$ Hz, 4 H), 1.44 (s, 18 H). Anal. ($\text{C}_{21}\text{H}_{33}\text{I}_2\text{N}_4\text{O}_9\text{P}$), C, H, N, P.

Similar treatment of ester **Ib-14E** with TFA gave diacid **Ib-14P** (97%) as a yellow foam. ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.90 (t, $J = 5.6$ Hz, 1 H), 8.73 (d, $J = 2.8$ Hz, 1 H), 8.34 (d, $J = 2.8$ Hz, 1 H), 3.98 (q, $J = 6.4$ Hz, 2 H), 3.49 (after D_2O exchange t, $J = 5.6$ Hz, 2 H), 3.45 (t, $J = 7.8$ Hz, 4 H), 3.29 (t, $J = 7.7$ Hz, 4 H). HRMS (FAB) Calcd. for $\text{C}_{13}\text{H}_{18}\text{I}_2\text{N}_4\text{O}_9$ $[\text{M}+\text{H}]^+$ m/z 658.3911; found 658.3907. Treatment of diacid **Ib-14P** with NaHCO_3 (2.0 equiv.) gave the disodium salt.

Example 34. 2-[(2-Iodoethyl)-2,4-dinitro-6-([2-(phosphonoxy)ethyl]amino)carbonyl]anilino]ethyl methanesulfonate (Ib-15P**).** Similar phosphorylation of alcohol **Ib-15** (1.68 g, 3.1 mmol) with di-*tert*-butyldiethylphosphoramidite (93%, 1.15 g, 4.5 mmol), followed by flash column chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1), and crystallization from EtOAc/petroleum ether, gave **Ib-15E** as a yellow solid (2.23 g, 97%): mp (EtOAc/petroleum ether) 109-111 $^\circ\text{C}$; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.98 (m, 1 H), 8.76 (d, $J = 2.8$ Hz, 1 H), 8.33 (d, $J = 2.8$ Hz, 1 H), 4.27 (m, 2 H), 4.00 (m, 2 H), 3.53 (m, 2 H), 3.46 (m, 4 H), 3.14 (s, 3 H), 1.43 (s, 18 H). ^{13}C NMR δ 165.5, 145.6, 145.2, 140.8, 135.6, 127.4, 122.4, 81.7, 67.5, 64.2, 55.4, 50.7, 39.9, 36.5, 29.3, 2.6. Anal. ($\text{C}_{22}\text{H}_{36}\text{I}\text{N}_4\text{O}_{12}\text{PS}$), C, H, N.

Similar treatment of **Ib-15E** (405 mg) with TFA (6 mL) and crystallization of the product from CH_2Cl_2 /petroleum ether gave diacid **Ib-15P** as a yellow solid (306 mg, 89%): mp 147-150 $^\circ\text{C}$; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.93 (m, 1 H), 8.74 (d, $J = 2.8$ Hz, 1 H), 8.36 (d, $J = 2.8$ Hz, 1 H), 4.27 (m, 2 H), 4.00 (m, 2 H), 3.46 (m, 6 H), 3.31 (m, 2 H), 3.12 (s, 3 H). ^{13}C NMR δ 165.5, 145.6, 145.2, 140.8, 135.7, 127.6, 122.3, 67.6, 63.3, 55.5, 50.7, 39.9, 36.5, 2.7. Anal. ($\text{C}_{14}\text{H}_{20}\text{I}\text{N}_4\text{O}_9\text{PS}$), C, H, N.

Example 35. 2-[(2-Chloroethyl)-2,4-dinitro-3-[[3-(phosphonoxy)propyl]amino]carbonyl]anilino]ethyl methanesulfonate (Ic-6P**).** Similar phosphorylation of **Ic-6**, followed by chromatography of the product on silica gel and elution with EtOAc/petroleum ether (from 1:2 to 1:1), gave 2-[(2-chloroethyl)-3-(7-*tert*-butoxy-9,9-dimethyl-7-oxido-6,8-dioxa-2-aza-7-phosphahex-1-anoyl)-2,4-dinitroanilino]ethyl methanesulfonate (**Ic-6E**) (98%) as a yellow solid: mp (EtOAc/petroleum ether) 98-102 $^\circ\text{C}$. ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.83 (t, $J = 5.6$ Hz, 1 H), 8.26 (d, $J = 9.4$ Hz, 1 H), 7.65 (d, $J = 9.4$ Hz, 1 H), 4.29 (t, $J = 5.3$ Hz, 2 H), 3.92 (q, $J = 6.7$ Hz, 2 H), 3.72-3.62 (m, 4 H), 3.62-3.55 (m, 2 H), 3.23 (q, $J = 6.5$ Hz, 2 H),

3.15 (s, 3 H), 1.79 (pent, $J = 6.7$ Hz, 2 H), 1.42 (s, 18 H). ^{13}C NMR δ 161.3, 146.4, 140.4, 137.6, 129.1, 127.6, 123.0, 81.2, 66.8, 64.1, 64.0, 52.9, 50.1, 41.4, 36.6, 35.9, 29.3. Anal. ($\text{C}_{23}\text{H}_{38}\text{ClN}_4\text{O}_{12}\text{PS}$) C, H, N.

- 5 Similar treatment of ester **Ic-6E** with TFA gave diacid **Ic-6P** (84%) as a yellow solid: mp (EtOAc/ CH_2Cl_2) 98-102 °C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.84 (t, $J = 5.7$ Hz, 1 H), 8.26 (d, $J = 9.4$ Hz, 1 H), 7.65 (d, $J = 9.4$ Hz, 1 H), 4.28 (t, $J = 5.3$ Hz, 2 H), 3.88 (q, $J = 6.8$ Hz, 2 H), 3.72-3.62 (m, 4 H), 3.53 (after D_2O exchange, t, $J = 6.0$ Hz, 2 H), 3.23 (q, $J = 6.6$ Hz, 2 H), 3.15 (s, 3 H), 1.76 (pent, $J = 6.7$ Hz, 2 H). Anal. ($\text{C}_{15}\text{H}_{22}\text{ClN}_4\text{O}_{12}\text{PS}$) C, H, N.

10

Example 36. 2-({3-[Bis(2-bromoethyl)amino]-2,6-dinitrobenzoyl}amino)ethyl dihydrogen phosphate (**Ic-8P**). Similar phosphorylation of alcohol **Ic-8** (1.41 g, 2.83 mmol) with di-*tert*-butyldiethylphosphoramidite (93%, 1.25 g, 5.0 mmol), followed by flash column
 15 chromatography on silica gel, eluting with EtOAc/petroleum ether (1:1), gave **Ic-8E** as a yellow solid (1.77 g, 91%): mp (EtOAc/petroleum ether) 112-114 °C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.86 (m, 1 H), 8.24 (d, $J = 9.4$ Hz, 1 H), 7.63 (d, $J = 9.4$ Hz, 1 H), 3.92 (m, 2 H), 3.70 (m, 4 H), 3.60 (m, 4 H), 3.22 (m, 2 H), 1.78 (m, 2 H), 1.41 (s, 18 H). ^{13}C NMR δ 161.4, 145.9, 139.9, 137.3, 129.2, 127.8, 122.5, 81.3, 64.1, 52.5, 35.9, 30.1, 29.4, 29.1. Anal. ($\text{C}_{22}\text{H}_{35}\text{Br}_2\text{N}_4\text{O}_9\text{P}$), C, H, N.

20

Similar treatment of **Ic-8E** (900 mg) with TFA (10 mL) gave diacid **Ic-8P** as a yellow foam (754 mg, 100%): ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.83 (m, 1 H), 8.24 (d, $J = 9.4$ Hz, 1 H), 7.63 (d, $J = 9.4$ Hz, 1 H), 3.86 (m, 2 H), 3.73 (m, 4 H), 3.60 (m, 4 H), 3.22 (m, 2 H), 1.76 (m, 2 H). ^{13}C
 25 NMR δ 161.3, 145.9, 140.1, 137.4, 129.2, 127.6, 122.5, 62.9, 52.5, 36.0, 30.0, 29.3. HRMS (FAB) calcd for $\text{C}_{14}\text{H}_{20}^{79}\text{Br}_2\text{N}_4\text{O}_9\text{P}$. $[\text{M}+\text{H}]^+$ m/z 576.9335, found 576.9326.

25

Example 37. 2-[(2-Bromoethyl)-2,4-dinitro-3-[[[2-(phosphonoxy)ethyl]amino]-carbonyl]anilino]ethyl methanesulfonate (**Ic-12P**). Similar phosphorylation of **Ic-12**,
 30 followed by chromatography of the product on silica gel and elution with EtOAc/petroleum ether (from 1:2 to 1:1), gave (**Ic-12E**) (99%) as a yellow solid: mp (EtOAc/petroleum ether) 82-86 °C (dec). ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 9.00 (t, $J = 5.6$ Hz, 1 H), 8.26 (d, $J = 9.4$ Hz, 1 H),

7.65 (d, $J = 9.4$ Hz, 1 H), 4.28 (t, $J = 5.3$ Hz, 2 H), 3.92 (q, $J = 6.7$ Hz, 2 H), 3.72-3.62 (m, 4 H), 3.62-3.55 (m, 2 H), 3.23 (q, $J = 6.5$ Hz, 2 H), 3.15 (s, 3 H), 1.42 (s, 18 H). Anal.

(C₂₂H₃₆BrN₄O₁₂PS) C, H, N.

- 5 Similar treatment of ester **Ic-12E** with TFA gave diacid **Ic-12P** (100%) as a yellow solid: mp (EtOAc/CH₂Cl₂) 93-97 °C; ¹H NMR [(CD₃)₂SO] δ 8.99 (t, $J = 5.7$ Hz, 1 H), 8.26 (d, $J = 9.4$ Hz, 1 H), 7.65 (d, $J = 9.4$ Hz, 1 H), 4.28 (t, $J = 5.3$ Hz, 2 H), 3.88 (q, $J = 6.8$ Hz, 2 H), 3.72-3.62 (m, 4 H), 3.53 (after D₂O exchange, t, $J = 6.0$ Hz, 2 H), 3.23 (q, $J = 6.6$ Hz, 2 H), 3.15 (s, 3 H). Anal. (C₁₄H₂₀BrN₄O₁₂PS) C, H, N.

10

Example 38. 2-[(2-Bromoethyl)-2,4-dinitro-3-[[[3-(phosphonoxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate (**Ic-13P**). Similar phosphorylation of **Ic-13**, followed by chromatography of the product on silica gel and elution with CH₂Cl₂/EtOAc (1:3), gave 2-[(2-bromoethyl)-3-(7-*tert*-butoxy-9,9-dimethyl-7-oxido-6,8-dioxa-2-aza-7-

- 15 phosphahex-1-ano-1-yl)-2,4-dinitroanilino]ethyl methanesulfonate (**Ic-13E**) (70%) as a yellow solid: mp (CH₂Cl₂/i-Pr₂O) 95-96 °C (dec). ¹H NMR [(CD₃)₂SO] δ 8.83 (t, $J = 5.6$ Hz, 1 H), 8.26 (d, $J = 9.4$ Hz, 1 H), 7.65 (d, $J = 9.4$ Hz, 1 H), 4.28 (t, $J = 5.3$ Hz, 2 H), 3.92 (q, $J = 6.7$ Hz, 2 H), 3.72-3.62 (m, 4 H), 3.62-3.55 (m, 2 H), 3.23 (q, $J = 6.5$ Hz, 2 H), 3.15 (s, 3 H), 1.79 (pent, $J = 6.7$ Hz, 2 H), 1.42 (s, 18 H). Anal. (C₂₃H₃₈BrN₄O₁₂PS) C, H, N, P.

20

Similar treatment of ester **Ic-13E** with TFA gave diacid **Ic-13P** (98%) as a hygroscopic yellow solid. ¹H NMR [(CD₃)₂SO] δ 8.84 (t, $J = 5.7$ Hz, 1 H), 8.26 (d, $J = 9.4$ Hz, 1 H), 7.65 (d, $J = 9.4$ Hz, 1 H), 4.28 (t, $J = 5.3$ Hz, 2 H), 3.88 (q, $J = 6.8$ Hz, 2 H), 3.72-3.62 (m, 4 H), 3.53 (after D₂O exchange, t, $J = 6.0$ Hz, 2 H), 3.23 (q, $J = 6.6$ Hz, 2 H), 3.15 (s, 3 H), 1.76 (pent, $J = 6.7$ Hz, 2 H). HRMS (FAB) calcd for C₁₅H₂₃⁷⁹BrN₄O₁₂PS [M+H]⁺ m/z 592.9954; found 592.9956. Treatment of diacid **Ic-13P** with NaHCO₃ (1:0 equiv) gave the monosodium salt.

25

Example 39. 2-[(2-Iodoethyl)-2,4-dinitro-3-[[[3-(phosphonoxy)propyl]amino]-carbonyl]anilino]ethyl methanesulfonate (**Ic-15P**). Similar phosphorylation of **Ic-15**,

30

followed by chromatography of the product on silica gel and elution with CH₂Cl₂/EtOAc (1:3), gave 2-[(2-iodoethyl)-3-(7-*tert*-butoxy-9,9-dimethyl-7-oxido-6,8-dioxa-2-aza-7-phosphahex-1-ano-1-yl)-2,4-dinitroanilino]ethyl methanesulfonate (**Ic-15E**) (58%) as a yellow

solid: mp (EtOAc/iPr₂O) 90-100 °C. ¹H NMR [(CD₃)₂SO] δ 8.86 (t, *J* = 5.6 Hz, 1 H), 8.25 (d, *J* = 9.4 Hz, 1 H), 7.63 (d, *J* = 9.5 Hz, 1 H), 4.27 (t, *J* = 5.2 Hz, 2 H), 3.91 (q, *J* = 6.7 Hz, 2 H), 3.67 (t, *J* = 5.2 Hz, 2 H), 3.60 (t, *J* = 7.1 Hz, 2 H), 3.26-3.17 (after D₂O exchange, m, partially obscured, 2 H), 3.23 (q, *J* = 6.5 Hz, 2 H), 3.15 (s, 3 H), 1.78 (pent, *J* = 6.6 Hz, 2 H). Anal.

5 (C₂₃H₃₈IN₄O₁₂PS) C, H, N, P.

Similar treatment of ester **Ic-15E** with TFA gave diacid **Ic-15P** (97%) as a hygroscopic yellow

solid: mp (CH₃CN/EtOAc) 84-86 °C. ¹H NMR [(CD₃)₂SO] δ 8.90 (t, *J* = 5.6 Hz, 1 H), 8.24 (d, *J* = 9.4 Hz, 1 H), 7.57 (d, *J* = 9.5 Hz, 1 H), 4.25 (t, *J* = 5.2 Hz, 2 H), 3.81 (after D₂O

10 exchange, q, *J* = 6.7 Hz, 2 H), 3.62 (after D₂O exchange, t, *J* = 5.2 Hz, 2 H), 3.56 (t, *J* = 7.1 Hz, 2 H), 3.26 (t, *J* = 6.9 Hz, 2 H), 3.20 (q, *J* = 6.5 Hz, 2 H), 3.09 (s, 3 H), 1.73 (pent, *J* = 6.6 Hz, 2 H). HRMS(FAB) calcd for C₁₅H₂₂IN₄O₁₂PS (MH⁺) *m/z* 640.9816. Found; 640.9795. Anal. (C₁₅H₂₂IN₄O₁₂PS) C, H.

15

Table 2. Combustion analysis data for new compounds of Tables 1a and 1b

| No | Found | | | | Calculated | | | |
|---------------|-------|-----|------|-----------|------------|-----|------|-----------|
| | C | H | N | other | C | H | N | other |
| IIa-1 | 44.5 | 3.9 | 18.6 | | 44.6 | 4.1 | 18.9 | |
| IIa-3 | 41.3 | 4.3 | 13.7 | 17.4 (Cl) | 41.1 | 4.4 | 13.7 | 17.3 (Cl) |
| IIa-7 | 32.6 | 3.3 | 11.6 | 33.3 (Br) | 32.3 | 3.3 | 11.6 | 33.0 (Br) |
| IIa-7s | 33.4 | 3.7 | 7.8 | | 32.5 | 3.7 | 8.1 | |
| IIa-8 | 33.9 | 3.6 | 11.4 | 32.1 (Br) | 33.8 | 3.6 | 11.3 | 32.1 (Br) |
| IIa-9 | 35.5 | 3.8 | 10.7 | 31.2 (Br) | 35.2 | 3.9 | 10.9 | 31.2 (Br) |
| IIa-14 | 27.3 | 2.6 | 9.6 | 43.8 (I) | 27.0 | 2.8 | 9.7 | 43.9 (I) |
| IIb-1 | 51.2 | 5.7 | 15.9 | | 51.3 | 5.7 | 15.9 | |
| IIb-3 | 41.6 | 4.5 | 13.6 | 17.1 (Cl) | 41.1 | 4.4 | 13.7 | 17.3 (Cl) |
| IIb-7 | 32.9 | 3.3 | 11.5 | 33.3 (Br) | 32.3 | 3.3 | 11.6 | 33.0 (Br) |
| IIb-7a | 35.3 | 3.8 | 10.9 | | 35.2 | 3.9 | 10.9 | |
| IIb-8 | 34.9 | 3.7 | 11.3 | 32.3 (Br) | 33.8 | 3.6 | 11.3 | 33.3 (Br) |

| | | | | | | | | |
|---------------|------|-----|------|---------|------|-----|------|---------|
| IIb-14 | 27.8 | 3.1 | 9.5 | | 27.0 | 2.8 | 9.7 | |
| IIc-12 | 33.8 | 3.7 | 11.0 | | 33.7 | 3.8 | 11.2 | |
| IIc-13 | 35.4 | 3.9 | 11.0 | | 35.2 | 4.1 | 10.9 | |
| IIc-14 | 36.7 | 4.5 | 10.2 | | 36.4 | 4.4 | 10.6 | |
| Ib-7E | 37.7 | 4.9 | 8.3 | 4.6 (P) | 37.3 | 4.9 | 8.3 | 4.6 (P) |
| Ib-2E | 44.8 | 6.2 | 9.0 | 5.1 (P) | | | | |
| Ib-14E | 32.9 | 4.2 | 7.2 | 3.8 (P) | 32.7 | 4.3 | 7.3 | 4.0 (P) |
| Ia-3E | 44.2 | 5.9 | 9.3 | | 43.9 | 5.9 | 9.3 | |
| Ia-8E | 38.5 | 5.0 | 8.2 | | 38.3 | 5.1 | 8.1 | |
| Ic-13E | 39.0 | 5.4 | 8.9 | 4.4 (P) | 39.2 | 5.4 | 7.9 | 4.4 (P) |
| Ic-15E | 37.0 | 5.0 | 7.3 | 4.2 (P) | 36.7 | 5.1 | 7.5 | 4.1 (P) |
| Ic-15P | 28.1 | 3.5 | | | 28.3 | 3.5 | | |

Representative alcohols of Formula I (listed in Table 1a) show selective cytotoxicity towards human cancer cell lines transfected with either the *E. coli* nitroreductase cDNA (NTR) (Table 3, columns 2 and 3), or human cytochrome P450 reductase (P450R) under hypoxic conditions (Table 3, columns 4 and 5). In this table, sensitivity ratios are displayed to indicate the degree of selectivity for either NTR expression (column 3) or hypoxia (column 5). However, overexpression of P450R is not required for hypoxic selectivity.

IC₅₀ values are derived from cell proliferation experiments, following 4 hour drug exposure under a gas phase of either 20% oxygen or 0% oxygen (anoxia, achieved using an anaerobic chamber). Cells were grown under aerobic conditions for a further 5 days, and cell density estimated using the sulforhodamine B assay, to determine the concentration of prodrug required to inhibit growth to 50% of control.

Table 3. Selective cytotoxicities of representative examples of the alcohols of Table 1a

| No | Human colon (4 h) | | Human lung (4 h) | |
|--------|---|--|---|---|
| | WiDr (NTR ^{+/ve}) IC ₅₀ (μM) | WiDr WT:NTR IC ₅₀ Ratio | A549 (P450R ^{+/ve}) anoxia IC ₅₀ (μM) | A549 (P450R ^{+/ve}) 20% O ₂ /anoxia IC ₅₀ Ratio |
| IIa-1 | 5.2 | 34 | 3.7 | 28 |
| IIa-2 | 48 | 26 | 25 | 3.7 |
| IIa-3 | 47 | 36 | 54 | 23 |
| IIa-7 | 1.5 | 99 | 6.7 | 49 |
| IIa-7s | 9.3 | 35 | 2.1 | 109 |
| IIa-8 | 1.6 | 224 | 23 | 6.6 |
| IIa-9 | 6.4 | 58 | 22 | 9.4 |
| IIa-10 | 10 | 22 | - | - |
| IIa-11 | 11 | 9 | - | - |
| IIa-12 | 4.2 | 116 | 73 | 10 |
| IIa-13 | 5 | 90 | 32 | 18 |
| IIa-14 | 2.9 | 49 | 13 | 4.5 |
| IIb-1 | 61 | 2 | 384 | <1.3 |
| IIb-2 | 11.8 | 47 | 18 | 20 |
| IIb-3 | 13.6 | 59 | 30 | 9 |
| IIb-4 | 14 | 18 | - | - |
| IIb-5 | 13 | 19 | - | - |
| IIb-6 | 27 | 5 | - | - |
| IIb-7 | 0.3 | 61 | 0.8 | 56 |
| IIb-7a | 0.5 | 27 | 1.0 | 5.3 |
| IIb-8 | 0.4 | 13 | 1.1 | 24 |
| IIb-9 | 0.9 | 5 | 1.4 | 20 |
| IIb-10 | 0.9 | 2 | 2.3 | 11 |
| IIb-11 | 1.0 | 2 | 6.6 | 4.5 |
| IIb-12 | 0.4 | 48 | 0.28 | 133 |
| IIb-13 | 0.3 | 27 | 0.15 | 138 |
| IIb-14 | 0.8 | 12 | 1.0 | 27 |
| IIb-15 | 0.3 | 31 | 0.28 | 118 |
| IIc-7 | 10 | 46 | 3.9 | 40 |
| IIc-8 | 5.0 | 70 | 6.6 | 24 |
| IIc-9 | 31 | 6 | 7.3 | 21 |
| IIc-12 | 5.0 | 84 | 2.6 | 173 |
| IIc-13 | 4.3 | 95 | 4.5 | 134 |
| IIc-14 | 20 | 16 | 7.1 | 57 |

The activity of the phosphates as hypoxic cytotoxins is demonstrated by the data in Figure 1 for the representative example (**Ib-7P**). This employs an *in vivo* excision assay with the Rif-1 tumour, where the oxic tumour cells are sterilised using 15Gy of radiation, and the cytotoxicity of an agent against the remaining hypoxic cells can be quantitated. Unexpectedly, the activity of the phosphate **Ib-7P** is found to exceed that of its parent alcohol (**IIb-7**) at their respective maximum tolerated doses (**Ib-7P** = 750 $\mu\text{mol/kg}$; **IIb-7** = 1000 $\mu\text{mol/kg}$).

Figure 1. *In vivo* activity of **IIb-7** and its corresponding phosphate pre-prodrug **Ib-7P**, relative to the known hypoxic cytotoxin tirapazamine.

Figure 1a.

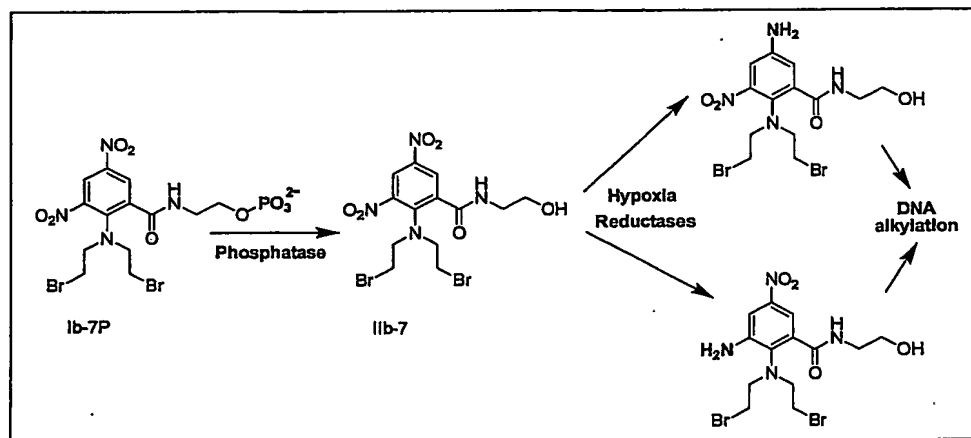
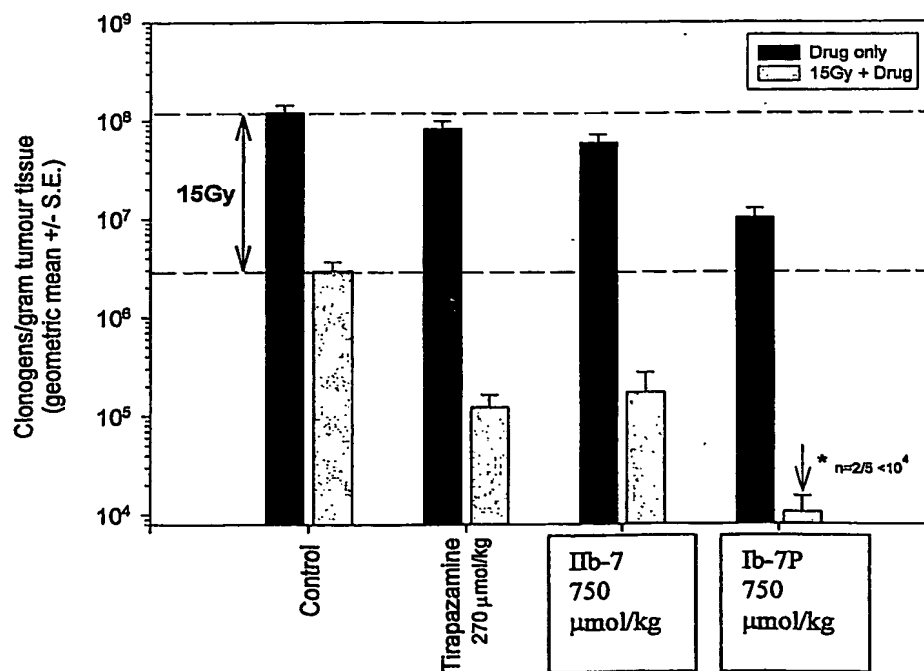


Figure 1b.

Excision assay method: 300-500mg Rif-1 xenograft \rightarrow 15Gy \rightarrow Single prodrug dose @ MTD \rightarrow 18hr \rightarrow excision \rightarrow disaggregate \rightarrow plate 10^2 - 10^5 cells \rightarrow 12 days \rightarrow colonies counted.



The notable activity of the phosphates of Formula I against hypoxic cells in human tumour xenografts is illustrated by the data of Table 4. In these experiments SiHa human cervical carcinoma cells were grown subcutaneously in CD-1 nude (immunodeficient) mice. The compounds were administered at doses corresponding to 75% or 20% of the maximum tolerated dose (MTD), 5 minutes after a whole body dose of ionising radiation (cobalt-60 gamma radiation) sufficient to sterilise the oxic cells (15 Gy). The tumours were excised 18 hours later, dissociated with a cocktail of proteases, and cell survival was determined using a clonogenic assay. The logarithms of cell kill were calculated from the difference in the numbers of clonogens per gram tumour tissue between treated and control tumours. In these experiments, some antitumour activity (tumour cell killing) was also observed when animals were treated with the compounds without irradiation at doses tending towards the MTD. (Table 4, column 4). This shows that the compounds also have antitumour activity as single agents.

Table 4. Activity of phosphates of Formula I against oxic and hypoxic cells in SiHa human tumour xenografts in nude mice. Compounds were administered as single i.p. doses in saline.

| No | MTD ($\mu\text{mol/kg}$) | 75% of MTD | | | 20% of MTD | |
|--------|-------------------------------|--------------------------------|--|---|--------------------------------|---|
| | | Dose ($\mu\text{mol/kg}$) | Log kill, cmpd only ^a Mean \pm sem (n) | Log kill, cmpd after radiation ^b | Dose ($\mu\text{mol/kg}$) | Log kill, cmpd after radiation ^b |
| Ia-8P | 1000 | 750 | ND ^c | ND | 200 | -0.01 \pm 0.19 |
| Ib-2P | 237 | 178 | ND | ND | 47 | -0.10 \pm 0.08 |
| Ib-2mP | 1780 | 1330 | 2.47 \pm 0.08 | \geq 3.47 \pm 0.17 | 356 | 2.56 \pm 0.37 |
| Ib-7P | 750 | 562 | 1.20 \pm 0.07 | 2.35 \pm 0.17 | 150 | 1.12 \pm 0.13 |
| Ib-12P | 1330 | 1000 | 1.32 \pm 0.30 | \geq 3.38 \pm 0.28 | 267 | 1.65 \pm 0.19 |
| Ib-14P | 562 | 422 | 0.92 \pm 0.06 | 1.93 \pm 0.21 | 113 | 0.64 \pm 0.11 |
| Ib-15P | 1330 | 1000 | 2.24 \pm 0.15 | \geq 3.27 \pm 0.13 | 267 | 1.74 \pm 0.23 |
| Ic-6P | 3160 | 2370 | 1.11 \pm 0.20 | \geq 2.62 \pm 0.17 | 632 | 1.64 \pm 0.30 |
| Ic-8P | 562 | 422 | ND | ND | 113 | 0.72 \pm 0.08 |
| Ic-12P | 1780 | 1330 | 1.04 \pm 0.29 | \geq 2.96 \pm 0.31 | 356 | 2.03 \pm 0.25 |
| Ic-13P | 3160 | 2370 | 2.36 \pm 0.28 | \geq 3.23 \pm 0.08 | 632 | 1.60 \pm 0.20 |
| Ic-15P | 1780 | 1333 | 2.27 \pm 0.17 | 2.80 \pm 0.40 | 356 | 1.81 \pm 0.23 |

| | | | | | | |
|------------------|------|------|-------------|------------|-----|-------------|
| Tirapazamine | 316 | 237 | -0.02 ±0.01 | 0.66 ±0.11 | 63 | -0.01 ±0.09 |
| Chlorambucil | 237 | 178 | 0.11 ±0.04 | 0.31 ±0.10 | 47 | 0.18 ±0.13 |
| Melphalan | 42.2 | 31.6 | ND | ND | 8.4 | 0.04 ±0.05 |
| Cyclophosphamide | 750 | 562 | ND | ND | 150 | 0.07 ±0.10 |

^a Relative to controls, in the same experiment, treated with vehicle (saline) only. Log kill = $\log_{10}(\text{clonogens/g tumour for control tumours}) - \log_{10}(\text{clonogens/g tumour for treated tumours})$.

^b Relative to the mean for radiation only, in the same experiment. Log kill = $\log_{10}(\text{clonogens/g tumour for radiation alone}) - \log_{10}(\text{clonogens/g tumour for tumours treated with radiation plus compound})$.

5 ^c None detected

A representative example of the phosphates of Formula I as NTR activated cytotoxins is provided in Figure 2. In the WiDr *in vivo* growth delay assay, xenografts containing mixtures of WiDr^{WT} and WiDr^{NTR} cells are grown to 300mm³ and treated with a single dose of prodrug at its MTD. Tumour growth is monitored over time and animals are euthanased when mean tumour volume >1600mm³. Data is presented as time to death. Unexpectedly, the activity of the phosphate (Ib-7P) is observed to exceed that of its parent alcohol (IIb-7), when administered at their respective maximum tolerated doses (750 µmol/kg). Ib-7P is superior to IIb-7 with respect to (i) time to first treatment failure (77-days vs. 17-days) and (ii) over-all survival (40% vs. 6%).

Figure 2. *In vivo* activity of IIb-7 and its corresponding phosphate pre-prodrug, Ib-7P, against human colon carcinoma xenografts containing 10% NTR+ve cells.

Figure 2a.

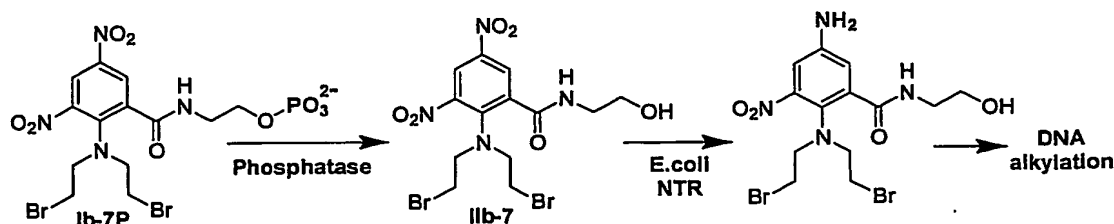
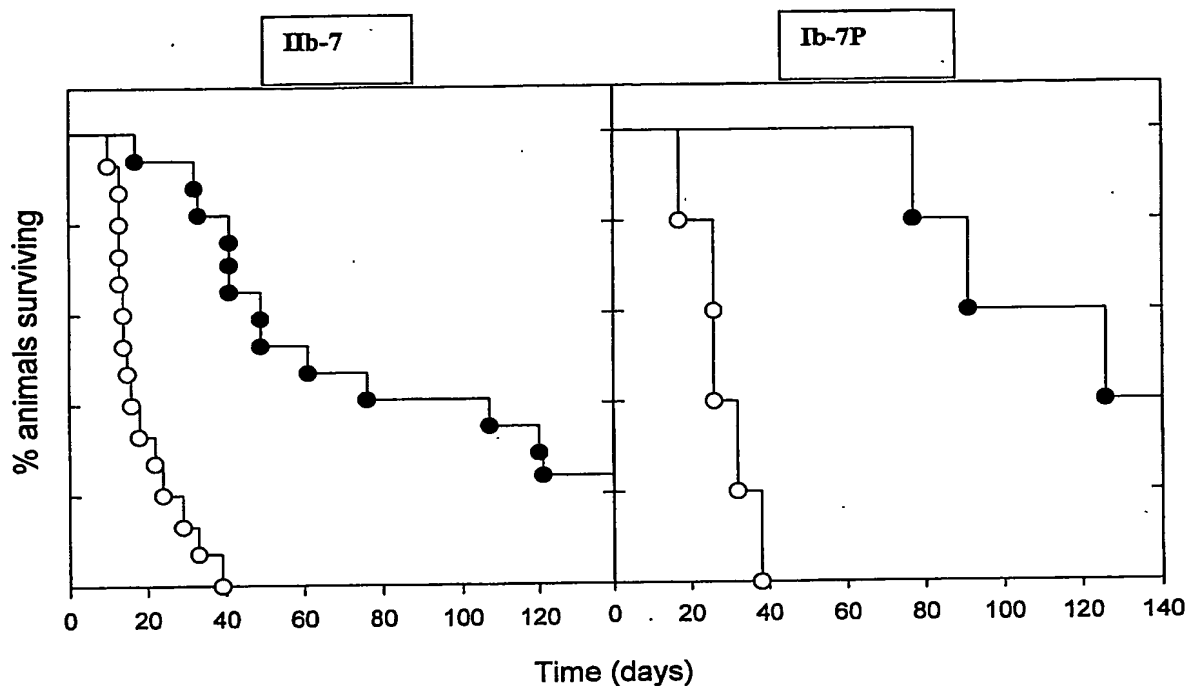


Figure 2b.



Legend: Open circles: wild-type WiDR human colon carcinoma xenografts
 Filled circles: WiDR cells transfected with the *E. coli* NTR gene.

Wherein the foregoing description reference has been made to reagents, or integers having known equivalents thereof, then those equivalents are herein incorporated as if individually set forth.

While this invention has been described with reference to certain embodiments and examples, it is to be appreciated that further modifications and variations may be made to embodiments and examples without departing from the spirit or scope of the invention.

AUCKLAND UNISERVICES LIMITED and
 WILLIAM ROBERT WILSON

By Their Attorneys
 BALDWIN